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205 ANNOUNCEMENTS

The Role of Granulocyte Transfusions in Treating and Preventing Infection[1]

Lowell S. Young[2,*]

No clinician who treats neutropenic cancer patients would deny the critical role of the granulocyte in the defense of the host against acute bacterial infection. Less clear is the role of these phagocytes against important fungal pathogens like aspergillus and candida species. Both of these opportunistic fungi are now commonly encountered in neutropenic subjects. Thus, when the technology became available in the mid-1960s for delivering granulocyte transfusions, considerable optimism was expressed that it would provide a new dimension to treating and preventing serious opportunistic infections (1). Nonetheless, more than a decade elapsed between the first reports of the therapeutic use of neutrophils harvested from human donors by centrifugal flow techniques and reports of controlled human trials demonstrating that such granulocyte transfusions were beneficial (2–5). Despite the appearance of these controlled studies, however, considerable doubt has arisen about the efficacy of and indications for such transfusions. The object of this review will be to critically examine the basis for claims of therapeutic efficacy, particularly in the light of new studies. I will also review the recent conclusions about prophylactic granulocyte studies, particularly the information on toxicity and complications.

As expressed by several workers (6–8), doubts about the potential efficacy of therapeutic granulocyte transfusions might be summarized in what could be called "the numbers game." Currently available techniques for harvesting granulocytes from healthy donors result in the collection of cells which are only a small fraction of what the normal host can produce for himself. The numbers of transfused granulocytes, 2.5×10^{10} cells (maximum), are roughly equal to the numbers of cells in the circulating granulocyte pool of an adult, but this represents only 5% of the daily production of granulocytes and 0.5% of the total body stores of granulocytes (8). Little wonder that removal of granulocytes from normal donors fails to lower the donor's wbc count and that posttransfusion increments in blood neutrophil counts in recipients are small and evanescent. An additional consideration is the ability of normal marrow to augment production of neutrophils when there is acute bacterial infection. An experimental animal under stress of infection has the capacity to augment the daily production of cells by a factor of 10–20 (6–7). Extrapolated to man, the infected neutropenic host who receives a single transfusion of granulocytes probably receives $< 5\%$ of what a normal bone marrow can produce daily when there is a serious systemic infection.

It is readily acknowledged that in the early days of granulocyte transfusions, when cells from patients with chronic myelocytic leukemia were given, significantly greater numbers of cells were transfused. As reviewed by McCullough and Quie (8), doses of $1–5 \times 10^{11}$ cells were possible when harvested from patients with high wbc counts secondary to chronic myelocytic leukemia. Even when cells from such sources were used, however, no adequately controlled studies were carried out to demonstrate therapeutic efficacy. For almost all wbc cell transfusions that are now performed, cells are obtained from normal donors by centrifugal flow or filtration leukapheresis techniques. Major questions have arisen about the quality of cells obtained by filtration techniques and whether they are prone to sequestration and agglutination in the pulmonary vascular bed.

With these doubts about the quality and quantity of transfused neutrophils, the burden of proof for their therapeutic efficacy has fallen on the results of controlled clinical trials. Four published randomized prospective studies of granulocyte transfusions that support the conclusion that they are effective have been reviewed in prior publications (9,10). Each of these affirmative studies contained small numbers of patients (< 20 in transfused and control groups) yet attained favorable statistical significance. Perhaps the best designed study, that of Herzig and colleagues (3), was restricted to patients with gram-negative rod bacteremia. The transfused patients, however, had shorter periods of neutropenia and more of them had bacteremic

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Escherichia coli infections than the control group. *Pseudomonas aeruginosa* infections are more difficult to treat than *E. coli* infections, and in the studies by Herzig et al (3) and Alavi et al (4) more patients with pseudomonas infections were randomized to the control group. Vogler and Winton (5) also concluded that therapeutic granulocytes were beneficial, yet analysis of their patients' survival 3 weeks after entry in the study showed no statistically significant difference between transfused and control groups.

More recently, a much larger trial of granulocyte transfusion therapy has been conducted in patients with documented infections at the University of California at Los Angeles (UCLA), and no significant difference in outcome and survival between transfused and control groups could be demonstrated (11). The wisdom of randomly allocating study patients with septicemic infections into transfused and control groups has been questioned. Herzig and colleagues (3) concluded that no neutropenic patient would recover from gram-negative rod bacteremia unless there was evidence for spontaneous bone marrow recovery or the patient was given therapeutic granulocyte transfusions. In contrast, there is ample evidence from the studies of Love et al (12), as well as from the randomized study of therapeutic transfusions carried out at the UCLA Center, that most individuals who have gram-negative bacteremia but no evidence for marrow recovery will respond to appropriate antimicrobial therapy. The concept that lack of marrow recovery mandates granulocyte transfusions forces us to scrutinize the criteria for this diagnosis. It is extremely difficult in any prospective study to really identify those individuals who lack evidence of marrow recovery. Clearly, it is a much easier ex post facto diagnosis, because the neutropenic patient who rapidly dies of sepsis will not have time for adequate marrow regeneration. In other studies of neutropenic cancer patients the appropriateness of antibiotic therapy has been a far more important factor influencing eventual outcome than whether a patient did or did not receive therapeutic granulocyte transfusions (13). In fact, the specific nature of the antibiotic therapy, as well as whether it was continued, augmented, or altered depending on the susceptibility of infecting organisms or whether it was chosen or altered on the basis of in vitro synergistic interactions, has never been clarified in any of the studies that have claimed to demonstrate the efficacy of therapeutic transfusions.

At the same time that considerable enthusiasm was being expressed for the efficacy of therapeutic transfusions, a major attempt was underway to assess the prophylactic efficacy of transfused granulocytes. The logic behind this approach was that, in view of the rather small numbers of cells that could be obtained for transfusion, approximately 10^{10} cells given daily might impact in the noninfected host and might prevent the onset of systemic infection when the bacterial inoculum is relatively small. Despite the enthusiastic report of the University of Washington Bone Marrow Transplant Group (14), questions must be raised about the appropriateness of their study design. For instance, patients were randomized to receive or not to receive prophylactic granulocytes, but some were already receiving antibiotics and were febrile upon entry in the study. Importantly, there was no difference in survival between transfused and control groups. It seems logical that the only valid study of prophylactic efficacy of granulocyte transfusions would be one in which patients without any evidence of infection (and not being treated with systemic antimicrobials) were randomized to receive or not to receive granulocytes. Such was the design of the study undertaken in bone marrow transplant patients at UCLA (15) and of the collaborative studies in patients with acute myelocytic leukemia summarized by Strauss et al (16). All of these studies do provide some support for the concept that prophylactic granulocytes can reduce the overall incidence of bacteremic infections. Of interest, however, is that in the University of Washington study, no prophylactic oral nonabsorbable antibiotics were used. In the UCLA prophylactic study on bone marrow transplant patients, oral nonabsorbable antibiotics were used routinely and there were no episodes of systemic gram-negative rod bacteremia in either control or transfused groups. That observation might provide a more cost-effective approach to the reduction of systemic gram-negative infections than the daily, expensive granulocyte transfusions. Of greater concern, however, were the observations of transfusion-associated pulmonary complications and cytomegalovirus (CMV) infections in recipients of prophylactic cells. A clear-cut difference in the incidence of CMV infections was seen in a prophylactic study at UCLA, with the group receiving granulocytes having significantly more evidence for CMV infections than those who did not receive granulocytes. A similar association was observed in a retrospective analysis of transfused patients seen at the University of Washington (17). What was disturbing from the multicenter study reported by Strauss et al (16) was the finding of a significantly increased incidence of lung infiltrates in the transfused group; unfortunately the etiology of these infiltrates was not often identified, although viral infection and leukoagglutinin reactions could not be excluded. The conclusion from that multicenter study was that in spite of reduction of infection in recipients of prophylactic granulocytes, the overall benefits were greatly outweighed by complications and cost. Thus, there seems to be strong basis for the view that there are no indications for prophylactic granulocytes in patients who we expect will have severe neutropenia secondary to antineoplastic therapy.

In this brief review, I chose not to analyze the issue

of cost-effectiveness of granulocyte transfusions, although the inference has been drawn that oral nonabsorbable antibiotics might well be comparable in prophylactic efficacy. It is easy to argue that the sizable cost of granulocytes plus limited evidence for efficacy of transfusions rarely justifies their present use. Does this mean that there are no clear-cut indications for use of granulocyte transfusions? Presently, it is my view that the use of therapeutic transfusions should be restricted to individuals who have a documented bacterial or fungal infection that is not responding to appropriate antimicrobial therapy. How long to wait before the initiation of granulocytes in that setting has not been resolved, but judging a response to antimicrobial treatment will usually require an observation period of some 3–5 days. Granulocytes should be given no less than daily and for a period of some 7–10 days before concluding that they do or do not have an effect. Ultimately, the issue of survival will clearly be related to control of the underlying disease. Individuals with persistent bone marrow aplasia and documented infection may not respond, particularly if the marrow is filled with malignant cells.

Are there areas in which further research must be undertaken to define a role for granulocyte transfusions? The issue of prophylactic transfusions might be reopened if there were a very inexpensive way for obtaining really large numbers of cells from HLA-compatible donors. Schiffer and colleagues (18) have pointed out that in their small controlled study there was a high incidence of hypersensitivity reactions with prophylactic transfusions. Since these reactions may be directed against HLA (and other) antigens, prophylactic granulocytes may jeopardize the efficacy of thrombocytes given either prophylactically or therapeutically. The use of HLA-matched donors and recipients would place an enormous logistic burden on blood banking facilities, but such a study is feasible. The logistic problems would be further increased by requiring that donors of both granulocytes and platelet products be seronegative against CMV, but this too would be a worthwhile subject for investigation.

In summary, physicians involved in the care of cancer patients and other compromised hosts should accept the responsibility for demonstrating both the efficacy and cost-effectiveness of any measures that they strongly advocate. Clearly, granulocyte transfusions are an approach where an initial demonstration of therapeutic efficacy has become clouded by better controlled studies with larger numbers of patients. At the same time, alternative developments in the management of the compromised host have provided less expensive approaches to the prevention of infection while the complications of prophylactic transfusions have led to the virtual abandonment of their use.

REFERENCES

1. Freireich EJ, Levin RH, Whang J, et al. Function and fate of transfused leukocytes from donors with chronic myelocytic leukemia in leukopenic recipients. Ann NY Acad Sci 113:1081–1087, 1965.
2. Higby DJ, Yates JW, and Henderson ES. Filtration leukapheresis for granulocyte transfusion therapy. N Engl J Med 292:761–766, 1975.
3. Herzig RH, Herzig GP, Graw RG, Jr, et al. Successful granulocyte transfusion therapy for gram-negative septicemia. N Engl J Med 296:701–705, 1977.
4. Alavi JB, Root RK, Djerassi I, et al. A randomized clinical trial of granulocyte transfusions for infection in acute leukemia. N Engl J Med 296:706–711, 1977.
5. Vogler WR, and Winton EF. A controlled study of the efficacy of granulocyte transfusions in patients with neutropenia. Am J Med 63:548–555, 1977.
6. Boggs DR. Transfusion of neutrophils as prevention or treatment of infection in patients with neutropenia. N Engl J Med 290:1055–1062, 1974.
7. ———. Neutrophils in the blood bank. N Engl J Med 296:748–754, 1979.
8. McCullough J, and Quie PG. Granulocyte transfusion: a current appraisal. *In* Infection in the Compromised Host, 2nd ed (Allen JC, ed). Williams and Wilkins, Baltimore, 1981, pp 1–36.
9. Berdischewsky M, and Young LS. Infectious complications of neoplastic disorders and their management. *In* Current Topics in Immunology (Franklin EC, ed). New York, Elsevier-North Holland Press, 1979, pp 307–338.
10. Young LS. Present status of granulocyte transfusions in the treatment or prevention of human infection. *In* Microbiology—1981 (Schlessinger W, ed). Washington, DC, American Society for Microbiology, pp 181–183.
11. Winston DJ, Ho W, and Gale RP. Therapeutic granulocyte transfusions for documented infections. Ann Intern Med 97:509–515, 1982.
12. Love LJ, Schimpff SC, Schiffer CA, et al. Improved prognosis for granulocytopenic patients with gram-negative rod bacteremia. Am J Med 68:643–648, 1980.
13. Young LS, Meyer-Dudnik DV, Hindler J, et al. Aminoglycosides in the treatment of bacteremic infections in the immunocompromised host. J Antimicrob Chemother 8 (suppl A):121–132, 1981.
14. Clift RA, Sanders JE, Thomas ED, et al. Granulocyte transfusions for the prevention of infection in patients receiving bone marrow transplants. N Engl J Med 298:1052–1057, 1978.
15. Winston DJ, Ho WG, Young LS, et al. Prophylactic granulocyte transfusions during human bone marrow transplantation. Am J Med 68:893–897, 1980.
16. Strauss RG, Connett JE, Gale RP, et al. A controlled trial of prophylactic granulocyte transfusions during initial induction chemotherapy for acute myelogenous leukemia. N Engl J Med 305:597–603, 1981.
17. Hersman J, Meyers JD, Thomas ED, et al. The effect of granulocyte transfusions on the incidence of cytomegalovirus infection after allogeneic marrow transplantation. Ann Intern Med 96:149–152, 1982.
18. Schiffer CA, Aisner J, Daly A, et al. Alloimmunization following prophylactic granulocyte transfusion. Blood 54:766–774, 1979.

Granulocyte Transfusion Therapy[1,2]

Charles A. Schiffer[3,*]

There is rather compelling logic suggesting the potential efficacy of granulocyte transfusion therapy in the management of infections in patients with marrow aplasia. The incidence of infection does not increase significantly in such patients until there are virtually no (< 100–200/μl) circulating granulocytes, indicating the clinical importance of even small numbers of granulocytes in preventing infection (1,2). Perhaps more critical is that when infection does occur in such patients, a number of antibiotic trials have demonstrated > 90% recovery from infection when minimal rises (< 100/μl) in granulocyte counts develop (2–6). Controlled trials in animals (7,8), multiple well-described observations of clear-cut benefits in individual patients (9–13), and controlled trials conducted in the mid-1970s in man (14–17) demonstrated that granulocyte transfusion could be of assistance in eradicating infections in certain clinical circumstances. Although none of these controlled studies were faultless, particularly because of the small numbers of patients with a wide variety of clinical situations analyzed [for more details, *see* recent critical reviews (18–20)], the weight of evidence seemed to clearly be in favor of the use of granulocyte transfusions in infected, granulocytopenic patients not responding to antibiotic therapy.

Indeed, this is precisely what happened in the late 1970s in clinical practice. Proliferation of relatively safe and mechanically simple cytopheresis machines in regional and hospital blood centers allowed widespread availability of granulocyte transfusion which theretofore had been limited to specialized centers. Increasing numbers of hematologists and oncologists treating larger numbers of patients more intensively resulted in a marked increase in the utilization of granulocyte transfusions in community hospitals and university settings. In the last few years, however, the use of granulocyte transfusions both in leukemia treatment centers and in the community has decreased substantially. This is undoubtedly related in part to the improved results of intensive induction therapy for acute leukemia. Remission is now achieved in approximately 70% of patients, with periods of aplasia which range from 14 to 28 days. In addition, remission generally occurs with one course of therapy (21). Because of this, if patients do develop serious infections it is often at the end of induction, at a time when marrow recovery is likely to occur, so that granulocyte transfusions are not needed. In addition, the widespread use of trimethoprim-sulfamethoxazole as prophylaxis against infection may also contribute to this decreased utilization (22,23).

I suspect also, however, that many clinicians have decreased or abandoned the use of granulocyte transfusion because of skepticism about the efficacy of the transfusions that they have administered. The significant costs of the transfusions as well as the associated side effects, particularly transfusion reactions, also may influence this attitude. Skepticism of this sort is certainly not a new event in the history of granulocyte transfusion therapy. Both the literature and the meetings devoted to the subject have frequently been quite contentious, with "the believers" (of whom I am one) stressing their clinical experience and the published controlled studies while the critics tended to legitimately focus on the difficulties of studying the end results of granulocyte transfusion. The publication of this commentary as well as another recent, large, editorial-type review of this subject (24) reflects this ongoing ambivalence. There are two major factors which I believe are often ignored or poorly appreciated by both the providers of granulocytes from the blood banks and the clinicians who utilize this therapy. This commentary will focus on these considerations, ie, dose of cells and histocompatibility factors, in an attempt to put the use of granulocyte transfusion therapy in proper perspective.

It is a somewhat remarkable fact that both the quality

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and the dose of granulocytes administered have been given minimal attention in many centers. Currently, the "quality" of granulocytes administered is probably not the most significant issue. Almost all blood centers obtain granulocytes using differential centrifugation cytopheresis machines which provide a functionally and morphologically normal granulocyte product (11,25,26). Nonetheless, granulocytes deteriorate rapidly during storage (27,28), and it is possible that transport and storage factors can result in an inferior product from certain centers. The issue of granulocyte function was more critical in the 1970s when collection in many centers was by filtration leukapheresis. This was a relatively inexpensive and efficient means of collection, but the function and morphology of the final granulocyte preparation varied widely according to technique (11,29–31). Although clinical efficacy could be demonstrated in some laboratories, the transfusion of these cells clearly resulted in an incidence of transfusion reactions and other recipient side effects (11,12,32). Because of these factors as well as the occurrence of occasionally severe donor side effects (33,34), this technique has been abandoned in most centers in this country. Nonetheless, many clinicians formed their personal impressions of the efficacy of granulocyte transfusion using granulocytes obtained by filtration leukapheresis.

The issue of granulocyte dose is considerably more important. To put this issue in perspective, one should recall that normal granulocyte turnover in an adult is approximately 10^{11} cells/day. This number can increase twofold to fourfold with stress of infection (35). Granulocyte yields can vary widely according to the cytopheresis technique utilized. All differential centrifugation machines must use a rouleauxing agent such as hydroxyethyl starch (36) in order to effectively separate granulocytes from rbcs, which have approximately the same density. Both discontinuous-flow (37,38) and continuous-flow (39,40) machines are available. Although the former apparatus can be more efficient in granulocyte separation, the volume of blood that can be processed is considerably smaller, and hence the overall yields tend to be lower. Donor premedication with corticosteroids can substantially increase yields by 100%–200% by inducing granulocytosis in the donor (36,41,42). Thus a wide range of yields can be achieved depending on the technique utilized. The dose commonly used can therefore vary from 5×10^9 cells/transfusion (discontinuous-flow centrifugation without steroid premedication) to $25–35 \times 10^9$ cells/transfusion (continuous-flow centrifugation with steroid premedication). It should be noted that the higher dose level is still tenfold less than normal granulocyte production. This is likely to be the case particularly in hospitals that receive their granulocytes from regional blood centers. For a variety of practical reasons as well as difficulties in following up donors who have received medications, many if not most regional blood centers are reluctant to premedicate donors with corticosteroids. In addition, at least until recently, most of these blood centers utilized intermittent centrifugation machines (Haemonetics model 30, Haemonetics Corp, Natick, MA). Thus, it is likely that most clinicians in this country utilizing granulocyte transfusion therapy utilize doses that are quite low when compared to normal granulocyte production.

The importance of the dose of granulocytes administered was recognized at least 20 years ago in the earliest granulocyte transfusion studies using donors with chronic myelogenous leukemia (CML). Freireich et al (13) described a dose-response relationship in severely infected patients receiving CML granulocytes. Similar findings were noted in other clinical studies (11,43) and in a well-conducted study in dogs in which the survival from *Pseudomonas aeruginosa* bacteremia was clearly related to the dose and quality of granulocytes administered (44). The same point was made in a study recently reported by Winston et al (45). In this randomized study, no benefit to infected, severely granulocytopenic patients could be demonstrated from the administration of a median dose of 5×10^9 granulocytes collected by a discontinuous centrifugation technique from non-steroid-premedicated donors. Although the author's conclusion is intended to cast doubt upon the efficacy of granulocyte transfusion, it is likely that this large well-conducted study only further indicates the importance of the dose of granulocytes administered. It is somewhat naive to expect serious infections, many of which are progressing despite antibiotic therapy, to respond to doses of granulocytes which are 1%–2% of the amount produced by normal subjects, and one must question this critical feature of this particular study.

The answer to the question of granulocyte dose is quite difficult, however. Even with improved technology and steroid premedication of the donor, one still can consistently procure only a fraction of what could be considered an ideal dose. Although it is sometimes possible to use multiple donors per patient, this cannot be routinely done because of limited numbers of donors and access to pheresis equipment. At our center we have recently "rediscovered the wheel" and have begun again to utilize donors with CML when they are available (46). It is possible to routinely obtain yields of $\geq 10^{11}$ myeloid cells because of the increased wbc count in these patients. In addition, because immature myeloid elements are also collected from CML donors, a sustained elevation in granulocyte count which can last from a few days to a week or more can occur in recipients of CML transfusions. This short-term "engraftment" can occur after as few as one or two transfusions and can be of striking clinical benefit. In our relatively small experience as well as the extensive experience reported by Benbunan (47), there have been no significant side effects following transfusion of CML granu-

locytes to nonalloimmunized recipients. In particular, we have not seen graft-versus-host disease even in those patients in whom continued division of myeloid elements occurred. Consideration should therefore be given to the use of CML granulocytes when such donors are available and in particular for patients with severe progressive infections.

In addition, because of the dosage problems imposed by the limitations of collection technology, it is preferable to begin granulocyte transfusions as soon as there is any clinical suspicion that antibiotic therapy is ineffective. It is usually possible for an experienced clinician to determine the likelihood of response to antibiotics within 24 hours of initiation of therapy; if possible, most decisions about the need for granulocyte transfusion should be made within 24–36 hours of the onset of infection. The site of infection, the type of organism, and the presence of bacteremia are clear-cut, important prognostic factors. Obviously, all infections are more poorly tolerated by patients with pre-existing cardiac, renal, or hepatic dysfunction. This is particularly true in elderly patients. Because of these factors, such patients should be considered for the earlier institution of granulocyte transfusion therapy prior to the occurrence of progressive infection unlikely to respond to any modality of therapy including the addition of moderate-dose granulocyte transfusion therapy.

In addition to dosage considerations, histocompatibility factors have also largely been ignored until recently in the evaluation of granulocyte transfusion therapy. Depending on the patient population, the number of previous transfusions, and the intensity of cytotoxic and immunosuppressive chemotherapy received, a variable percentage of potential recipients of granulocyte transfusion will be alloimmunized (ie, have preformed antibody against histocompatibility antigens on granulocytes and platelets). Approximately 40%–50% of potential recipients with leukemia will be alloimmunized, for example (48,49). It is well known that most febrile reactions following rbc transfusions are due to recipient antibody directed against leukocytes administered with the whole blood or packed rbcs. Severe febrile reactions were described in many recipients in the early granulocyte transfusion literature, almost certainly related to the larger number of cells administered with leukocyte transfusions to alloimmunized recipients (50,51). Although logic and accumulated knowledge about the adverse effects of antibody on transplants of any type would suggest that such reactions were associated with poor clinical results due to granulocyte destruction, it was difficult to prove this in human recipients. Until recently, therefore, most blood collection centers did not consider possible histocompatibility factors when selecting donors for recipients of granulocyte transfusions.

In granulocytopenic animal models it has been demonstrated that "random donor" granulocytes do not migrate to sites of infection in alloimmunized dogs. In these studies, posttransfusion granulocyte recovery and migration to skin windows (52) or to the cerebral spinal fluid in dogs with meningitis (53) were markedly reduced in alloimmunized animals compared to nonalloimmunized controls. In another model in which bacteremia was induced in granulocytopenic dogs, survival was also impaired in alloimmunized granulocyte transfusion recipients (54). Obviously it is difficult to perform comparable experiments in man. Until recently it has been difficult, if not impossible, to evaluate the results of individual granulocyte transfusions. Posttransfusion count increments, the traditional measurement of the "effectiveness" of any infused blood product, are almost invariably quite low, with absolute posttransfusion increments in the 100–300/μl range. Because of inaccuracies of counts and differential at these levels, posttransfusion count increments are totally unsatisfactory as a means of assessing results of granulocyte transfusion, although the literature is filled with attempts to use them as such. Evaluation of clinical response is invariably difficult because of the large numbers of factors that affect overall outcome in individual patients. Similar problems occur when evaluating the incidence of transfusion reactions.

Recently, however, the use of the radioisotope indium-111 oxine as a granulocyte label has enabled experiments to be done in man which have provided results parallel to those noted in animals. Indium-111 oxine does not damage granulocytes when cell labeling is done in vitro, and it is a gamma emitter that permits high-resolution scanning after the administration of small numbers (approximately 10^8) of labeled granulocytes (55). Two studies have been published (56,57) in which granulocytopenic patients with discrete areas of infection received labeled granulocytes from nonmatched donors, with follow-up scans done over the sites of infection. Migration of granulocytes to infected sites as early as 30 minutes after injection was noted in nonalloimmunized patients, indicating that careful pretransfusion matching is not important for such recipients (58). The results were quite different in alloimmunized patients, however. In our institution, 20 of 20 scans in nonalloimmunized patients were positive at known sites of infection, indicating granulocyte migration after infusion (57). In a group of alloimmunized patients identified clinically by their refractoriness to platelet transfusions along with a history of prior transfusion reactions, scans were positive in only three of 14 studies ($P < 0.001$). This indicates an absence of random-donor granulocyte migration to sites of infection in such patients and strongly suggests that nonmatched granulocyte transfusion administered to such patients is clinically ineffective.

A recent retrospective analysis of the outcome of granulocyte transfusions to alloimmunized patients is

consistent with these experiments demonstrating poorer results than were seen in nonalloimmunized patients (59). Because of the potential for donor morbidity and the significant potential for recipient side effects, it is quite clear that random-donor granulocyte transfusion should not be administered to such patients. It should be noted that histocompatibility data are available in only one of the controlled trials of therapeutic granulocyte transfusions which have been reported and obviously could have influenced the results of some of the other trials. In addition, histocompatibility factors almost certainly strongly influenced the results of many if not most of the granulocyte transfusions administered in this country over the last 5–10 years.

It is a question of what constitutes "a matched transfusion" for such patients which remains extremely problematic. Granulocytes have a number of different antigens on their surface (60) including antigens shared with other cells (ie, HLA) as well as antigens unique to granulocytes ("granulocyte-specific" antigens). At least four loci have been identified for granulocyte-specific antigens. These antigens are inherited independently of HLA antigens (61), have gene frequencies of 30%–70% (60) so that it is likely that donors will be mismatched for some of these antigens, and seem to be strongly expressed on granulocytes. Further complicating matters is the difficulty in reproducible granulocyte antibody testing in vitro. Although a variety of leukoagglutination assays have been utilized in many laboratories (60–62), the test has a subjective end point and is difficult to reliably perform. Granulocyte immunofluorescent testing, which measures the binding of antibody to granulocytes, may be a more reliable test (63). It has the disadvantage, however, of not distinguishing between antibody directed against HLA and granulocyte-specific antigens.

Although both of the indium-111 studies clearly demonstrate the ineffectiveness of random-donor granulocytes for alloimmunized patients, neither permits conclusions about which groups of antigens are most important for cross-matching purposes. This is due in part to the observation that most alloimmunized patients tend to have antibody detectable both by lymphocytotoxic (HLA) and leukoagglutinin cross-matching. Both studies contain examples of migration to infected sites despite positive donor-recipient lymphocytotoxic or leukoagglutinin cross-matches (56,57). Definitive advice about donor selection for alloimmunized patients, who can be recognized clinically by their failure to respond to platelet transfusion and serologically by the detection of lymphocytotoxic antibody, remains difficult. In our center we attempt to utilize compatible platelet donors as granulocyte donors, with a preference for family members, in the hope that granulocyte-specific antigens are more likely to be identical in the family setting. It may be helpful to monitor the platelet count increments after mixed platelet-granulocyte transfusions as a marker, albeit indirect, for compatibility. If significant transfusion reactions occur new donors are used, and if no apparent closely matched donors are available then transfusions are not administered. It is unusual to be able to identify large numbers of potentially compatible donors for alloimmunized patients, and therefore donors are often requested to donate repeatedly for such patients. This can represent a significant burden for some donors, and should be considered when a course of transfusion is initiated. In addition, it is unclear how frequently such donors can safely receive corticosteroid premedication. More definitive recommendations require the development of rapid, simpler, and more reproducible granulocyte cross-matching tests which can be shown to correlate with posttransfusion results. It is hoped that future advances in granulocyte serotyping, perhaps related to monoclonal antibody testing, will permit typing of granulocyte antigens in advance, allowing creation of donor files similar to what is available for HLA-matched platelet donation.

There are additional issues that are of importance and should briefly be mentioned. Although it has been demonstrated that prophylactic granulocyte transfusion therapy administered daily can in fact reduce the incidence of infection in both leukemia and bone marrow transplant patients, it has not been demonstrated that overall survival of these patients is improved (64,65). This is presumably related to successful treatment of acquired infections with antibiotic therapy and/or therapeutic granulocyte transfusions. It has also been demonstrated that the recipients of prophylactic granulocyte transfusion have an unacceptably high rate of transfusion reactions and subsequent alloimmunization to platelet transfusion (66,67). Because of the cost (68), the high rate of alloimmunization, and donor considerations, prophylactic granulocyte transfusion should be utilized only in investigational settings, if ever.

To date, the granulocyte transfusion literature has dealt almost entirely with the treatment of bacterial infections. There is relatively little published experience dealing with the treatment of fungal infection. It is clear that granulocyte transfusion can kill filamentous fungi, such as aspergillus species, as well as yeast organisms in vitro (69). In a dog model of systemic candida infection there is evidence that granulocyte transfusions can decrease the severity of the fungal infection (53,70). In infected humans, clinical improvement, with eradication of fungal infections, often occurs relatively promptly with marrow regeneration. It is likely that dosage considerations are of even greater importance than with bacterial infections, particularly since there are often delays in diagnosis and institution of antifungal therapy for most patients. Although further clinical studies are required, I feel that granulocyte transfusion therapy should be considered (although only with max-

imal doses of cells) in patients with nonresponsive fungal infections, in view of the overall poor prognosis of these infections. It should be emphasized, however, that even advanced fungal infections can often stabilize and improve with amphotericin B therapy, allowing time for marrow recovery with eventual clearing of infection (71).

Another issue with respect to the treatment of fungal infection is the possible adverse interaction between amphotericin B and granulocyte transfusion therapy. This interaction was characterized clinically by the acute onset of severe respiratory deterioration which contributed to death in five patients (72). Although other clinical reports have failed to confirm the initial description of increased pulmonary reactions in man (73), there are data in animals suggesting that both in vivo and in vitro exposure to amphotericin can promote aggregation of granulocytes which have been previously damaged in vitro, with resulting pulmonary sequestration (74). Until more data are available it would appear advisable to administer only fresh, centrifugation-prepared granulocytes to patients receiving amphotericin B. Transfusions should be administered slowly and monitored carefully with frequent chest X-rays. It should be emphasized, however, that current evidence does not permit the conclusion that concurrent amphotericin B administration and granulocyte transfusion therapy are contraindicated.

These issues, as well as the critical issues of dose and histocompatibility, all emphasize the complexities surrounding the proper use of granulocyte transfusion therapy. Granulocyte transfusion therapy clearly has been overused in many centers in the past and similarly has been inappropriately "abused" by detractors in blood banking, infectious disease, and hematology/oncology circles. As is common, the truth lies somewhere in between. Because of advances in antileukemia therapy and improvements in antibiotic usage, the overwhelming majority of granulocytopenic patients recover from infections with antibiotic therapy alone and do not require granulocyte transfusions. In addition, a significant percentage of patients refractory to antibiotics also have refractory tumors and would not be candidates for this intensive, costly medical care which has the potential for associated donor morbidity. Nonetheless, there remain a substantial number of patients with infections refractory to antibiotic therapy who could potentially benefit from the institution of granulocyte transfusion therapy if used properly. As indicated, dosage considerations and histocompatibility factors potentially limit the efficacy of transfusions in some such patients. Collection technology and immunologic monitoring of patients have improved recently, although not to the point that further investigations are no longer required. Granulocyte transfusion therapy cannot be considered a "routine" procedure, and a close consultative relationship must exist between the blood bank and the responsible clinicians so that the granulocyte transfusion products can be ordered or altered rapidly as demanded by changes in the individual patient's condition.

REFERENCES

1. Hahn DM, Schimpff SC, Fortner CL, et al. Infection in acute leukemia patients receiving oral nonabsorbable antibiotics. Antimicrob Agents Chemother 13:958–964, 1978.
2. Love LJ, Schimpff SC, Schiffer CA, et al. Improved prognosis for granulocytopenic patients with gram-negative bacteremia. Am J Med 68:643–648, 1980.
3. EORTC International Antimicrobial Therapy Project Group. Three antibiotic regimens in the treatment of infection in febrile granulocytopenic patients with cancer. J Infect Dis 137:14–29, 1978.
4. Keating MJ, Bodey GP, Valdivieso M, et al. A randomized comparative trial of three aminoglycosides—comparison of continuous infusions of gentamicin, amikacin and sisomicin combined with carbenicillin in the treatment of infections in neutropenic patients with malignancies. Medicine 58:159–170, 1979.
5. Klastersky J. Combinations of antibiotics for therapy of severe infections in cancer patients. Eur J Cancer 15:3–13, 1979.
6. Schimpff SC. Therapy of infection in patients with granulocytopenia. Med Clin North Am 61:1101–1118, 1977.
7. Epstein RB, Waxman FJ, Bennett BT, et al. Pseudomonas septicemia in neutropenic dogs. I. Treatment with granulocyte transfusions. Transfusion 14:51–57, 1974.
8. Dale DC, Reynolds HY, Pennington JE, et al. Experimental pseudomonas pneumonia in leukopenic dogs: comparison of therapy with antibiotics and granulocyte transfusions. Blood 47:869–876, 1976.
9. Eyre HJ, Goldstein IM, Perry S, et al. Leukocyte transfusions: function of transfused granulocytes from donors with chronic myelocytic leukemia. Blood 36:432–442, 1970.
10. McCredie KB, Freireich EJ, Hester JP, et al. Leukocyte transfusion therapy for patients with host-defense failure. Transplant Proc 5:1285–1289, 1973.
11. Aisner J, Schiffer CA, and Wiernik PH. Granulocyte transfusions: evaluation of factors influencing results and a comparison of filtration and intermittent centrifugation leukapheresis. Br J Haematol 38:121–129, 1978.
12. Schiffer CA, Buchholz DH, Aisner J, et al. Clinical experience with transfusion of granulocytes obtained by continuous flow filtration leukopheresis. Am J Med 58:373–381, 1975.
13. Freireich EJ, Levin RH, Whang J, et al. The function and fate of transfused leukocytes from donors with chronic myelocytic leukemia in leukopenic recipients. Ann NY Acad Sci 113:1081–1089, 1964.
14. Higby DJ, Yates JW, Henderson ES, et al. Filtration leukapheresis for granulocyte transfusion therapy. Clinical and laboratory studies. N Engl J Med 292:761–766, 1975.
15. Alavi JB, Root RK, Duerassi I, et al. A randomized clinical trial of granulocyte transfusions for infection in acute leukemia. N Engl J Med 296:706–711, 1977.
16. Herzig RH, Herzig GP, Graw RG, Jr, et al. Successful granulocyte transfusion therapy for gram-negative septicemia. A prospectively randomized controlled study. N Engl J Med 296:701–705, 1977.
17. Vogler WR, and Winton EF. A controlled study of the efficacy of granulocyte transfusions in patients with neutropenia. Am J Med 63:548–554, 1977.
18. Schiffer CA, and Aisner J. Rational approach to granulocyte transfusion therapy. *In* Contemporary Hematology/Oncology (LoBue J, and Silber R, eds). New York, Plenum Publishing Corp, 1980, pp 201–218.

19. Higby DJ. Controlled prospective studies of granulocyte transfusion therapy. Exp Hematol 5 (suppl 1):57–64, 1977.
20. Strauss RG, Therapeutic neutrophil transfusions. Are controlled studies no longer appropriate? Am J Med 65:1001–1006, 1978.
21. Gale RP. Advances in the treatment of acute myelogenous leukemia. N Engl J Med 300:1189–1199, 1979.
22. Gurwith MJ, Brunton JL, Lank BA, et al. A prospective controlled investigation of prophylactic trimethoprim/sulfamethoxazole in hospitalized granulocytopenic patients. Am J Med 66:248–256, 1979.
23. Wade JC, Schimpff SC, Hargadon MT, et al. A comparison of trimethoprim-sulfamethoxazole plus nystatin with gentamicin plus nystatin in the prevention of infections in acute leukemia. N Engl J Med 304:1057–1062, 1981.
24. International Forum. Granulocyte transfusions—an established or still an experimental therapeutic procedure? Vox Sang 38:40–58, 1980.
25. McCullough J, Weiblen BJ, Deinard AR, et al. In vitro function and post-transfusion survival of granulocytes collected by continuous-flow centrifugation and by filtration leukapheresis. Blood 48:315–326, 1976.
26. Glasser L, Huestis DW, and Jones JF. Functional capabilities of steroid-recruited neutrophils harvested for clinical transfusion. N Engl J Med 297:1033–1036, 1977.
27. McCullough J. Liquid preservation of granulocytes. Transfusion 20:129–137, 1980.
28. Price TH, and Dale DC. Neutrophil transfusion: effect of storage and of collection method on neutrophil blood kinetics. Blood 51:789–798, 1978.
29. Harris MB, Djerassi I, Schwartz E, et al. Polymorphonuclear leukocytes prepared by continuous-flow filtration leukapheresis: viability and function. Blood 44:707–713, 1974.
30. Klock JC, and Bainton DF. Degranulation and abnormal bactericidal function of granulocytes procured by reversible adhesion to nylon wool. Blood 48:149–161, 1976.
31. Wright DG, Kauffmann JC, Terpstra GK, et al. Mobilization and exocytosis of specific (secondary) granules by human neutrophils during adherence to nylon wool in filtration leukapheresis (FL). Blood 52:770–782, 1978.
32. Herzig GP, Bull MI, Lohrmann H-P, et al. Impaired transfusion response to granulocyte collected by filtration leucapheresis. *In* Leucocytes. Separation Collection and Transfusion. (Goldman JM, and Lowenthal RM, eds). London, Academic Press, 1975, pp 324–328.
33. Nusbacher J, Rosenfeld SI, MacPherson JL, et al. Nylon fiber leukapheresis: associated complement component changes and granulocytopenia. Blood 51:359–365, 1978.
34. Dahlke MB, Shah SL, Sherwood WC, et al. Priapism during filtration leukapheresis. Transfusion 19:482–486, 1979.
35. Craddock CG. Production, distribution and fate of granulocytes. *In* Hematology (Williams WJ, Beutler E, Erslev AJ, et al, eds). New York, McGraw Hill, 1972, pp 607–618.
36. Mishler JM, Higby DJ, Rhomberg W, et al. Hydroxyethyl starch and dexamethasone as an adjunct to leukocyte separation with the IBM blood cell separator. Transfusion 14:352–356, 1974.
37. Aisner J, Schiffer CA, Wolff JH, et al. A standardized technique for efficient platelet and leukocyte collection using the Model 30 Blood Processor. Transfusion 16:437–445, 1976.
38. Huestis DW, White RF, Price MJ, et al. Use of hydroxyethyl starch to improve granulocyte collection in the Latham blood processor. Transfusion 15:559–564, 1975.
39. Kalmin ND, and Grindon AJ. Pheresis with the IBM 2997. Transfusion 21:325–329, 1980.
40. Hester JP, Kellogg RM, Mulzet AP, et al. Principles of blood separation and component extraction in a disposable continuous-flow single-stage channel. Blood 54:254–268, 1979.
41. Winton EF, and Vogler WR. Development of a practical oral dexamethasone premedication schedule leading to improved granulocyte yields with the continuous-flow centrifugal blood cell separator. Blood 52:249–253, 1978.
42. Higby DJ, Mishler JM, Rhomberg W, et al. The effect of a single or double dose of dexamethasone on granulocyte collection with the continuous flow centrifuge. Vox Sang 28:243–248, 1975.
43. Higby DJ, Freeman A, Henderson ES, et al. Granulocyte transfusions in children using filter-collected cells. Cancer 38:1407–1413, 1976.
44. Appelbaum FR, Bowles CA, Makuch RW, et al. Granulocyte transfusion therapy of experimental Pseudomonas septicemia: study of cell dose and collection technique. Blood 52:323–331, 1978.
45. Winston DJ, Ho WG, Gale RP, et al. Therapeutic granulocyte transfusions for documented infections: a controlled trial in ninety-five infectious granulocytopenic episodes. Ann Intern Med, 1982.
46. Schiffer CA, Aisner J, Dutcher JP, et al. Short term engraftment following transfusion of leucocytes from patients with chronic myelogenous leukemia (CML). Am J Hematol. In press.
47. Benbunan M. Granulocyte transfusions—an established or still an experimental therapeutic procedure. Vox Sang 38:40–44, 1980.
48. Schiffer CA, Lichtenfeld JL, Wiernik PH, et al. Antibody response in patients with acute non-lymphocytic leukemia. Cancer 37:2177–2182, 1976.
49. Dutcher JP, Schiffer CA, Aisner J, et al. Alloimmunization following platelet transfusion: the absence of a dose response relationship. Blood 57:395–398, 1980.
50. Graw RG, Goldstein IM, Eyre HJ, et al. Histocompatibility testing for leucocyte transfusion. Lancet 77–78, 1970.
51. Goldstein IM, Eyre HJ, Terasaki PI, et al. Leukocyte transfusions: role of leukocyte alloantibodies in determining transfusion response. Transfusion 11:19–24, 1971.
52. Appelbaum FR, Trapani RJ, and Graw RG, Jr. Consequences of prior alloimmunization during granulocyte transfusion. Transfusion 17:460–464, 1977.
53. Chow HS, Sarpel SC, and Epstein RB. Pathophysiology of *Candida albicans* meningitis in normal, neutropenic, and granulocyte transfused dogs. Blood 55:546–551, 1980.
54. Westrick MA, Debelak-Fehir KM, and Epstein RB. The effect of prior whole blood transfusion on subsequent granulocyte support in leukopenic dogs. Transfusion 17:611–614, 1977.
55. Thakur ML, Lavender JP, Arnot RN, et al. Indium-111-labeled autologous leukocytes in man. J Nucl Med 18:1014–1021, 1977.
56. McCullough J, Weiblen BJ, Clay ME, et al. Effect of leukocyte antibodies on the fate in vivo of indium-111-labeled granulocytes. Blood 58:164–170, 1981.
57. Dutcher JP, Schiffer CA, Johnston GS, et al. The effect of histocompatibility factors on the migration of transfused ^{111}indium-labeled granulocytes. Blood 58(suppl):181a, 1981.
58. Dutcher JP, Schiffer CA, and Johnston GS. Rapid migration of ^{111}indium-labeled granulocytes to site of infection. N Engl J Med 304:586–589, 1981.
59. Dahlke MB, Keashen M, Alavi JB, et al. Granulocyte transfusions and outcome of alloimmunized patients with gram-negative sepsis. Transfusion 22:374–378, 1982.
60. Lalezari P. Neutrophil antigens: immunology and clinical implications. *In* The Granulocyte: Function and Clinical Utilization (Greenwalt TJ, and Jamieson GA, eds). New York, Alan R Liss, Inc, 1977, pp 209–225.
61. Schacter B, Preis P, Kadushin JM, et al. Family studies of neutrophil alloantigens in bone marrow transplantation. Tissue Antigens 16:267–273, 1980.
62. Ungerleider RS, Appelbaum FR, Trapani RJ, et al. Lack of predictive value of antileukocyte antibody screening in granulocyte transfusion therapy. Transfusion 19:90–94, 1979.
63. Verheugt FWA, von dem Borne AEG Kr, Decary F, et al. The detection of granulocyte alloantibodies with an indirect immunofluorescence test. Br J Haematol 36:533–544, 1977.

64. Clift RA, Sanders JE, Thomas ED, et al. Granulocyte transfusions for the prevention of infection in patients receiving bone-marrow transplants. N Engl J Med 298:1052–1057, 1978.
65. Mannoni P, Rodet M, Radeau E, et al. Granulocyte transfusion: efficiency of prophylactic granulocyte transfusions in care of patients with acute leukemia. *In* Blood Leucocytes: Function and Use in Therapy (Hogman CS, Lindahl-Kiessling K, and Wigzell H, eds). Stockholm, Almquist and Wiksell International, 1977.
66. Schiffer CA, Aisner J, Daly PA, et al. Alloimmunization following prophylactic granulocyte transfusion. Blood 54:766–774, 1979.
67. Strauss RG, Connett JE, Gale RP, et al. A controlled trial of prophylactic granulocyte transfusions during initial induction chemotherapy for acute myelogenous leukemia. N Engl J Med 305:597–638, 1981.
68. Rosenshein MS, Farewell VT, Price TH, et al. The cost effectiveness of therapeutic and prophylactic leukocyte transfusion. N Engl J Med 302:1058–1062, 1980.
69. Diamond RD, Krzesicki R, Epstein B, et al. Damage to hyphal forms of fungi by human leukocytes in vitro. Am J Pathol 91:313–323, 1978.
70. Ruthe RC, Anderson BR, Cunningham BL, et al. Efficacy of granulocyte transfusions in the control of systemic candidiasis in the leukopenic host. Blood 52:493–498, 1978.
71. Aisner J, Schimpff SC, and Wiernik PH. Treatment of invasive aspergillosis: relation of early diagnosis and treatment to response. Ann Intern Med 86:539–543, 1977.
72. Wright DG, Robichaud KJ, Pizzo PA, et al. Lethal pulmonary reactions associated with the combined use of amphotericin B and leukocyte transfusions. N Engl J Med 304:1185–1189, 1981.
73. Dana BW, Durie BGM, White RF, et al. Concomitant administration of granulocyte transfusions and amphotericin B in neutropenic patients: absence of significant pulmonary toxicity. Blood 57:90–94, 1981.
74. Boxer LA, Ingraham LM, Allen J, et al. Amphotericin-B promotes leukocyte aggregation of nylon-wool-fiber-treated polymorphonuclear leukocytes. Blood 58:518–522, 1981.

Comparisons of Carmustine, Procarbazine, and High-Dose Methylprednisolone as Additions to Surgery and Radiotherapy for the Treatment of Malignant Glioma[1,2]

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Within 3 weeks of definitive surgery, 609 patients with histologically demonstrated, supratentorial malignant glioma were randomized to receive, in addition to 6000 rads of radiotherapy, one of four treatment regimens: carmustine (BCNU), high-dose methylprednisolone, procarbazine, or BCNU plus high-dose methylprednisolone. We analyzed the data for the total randomized population and for the 527 patients (87% with glioblastoma multiforme) in whom the initial protocol specifications were met (the valid study group). Significantly longer survival was experienced by patients receiving procarbazine or BCNU alone compared to those receiving only high-dose methylprednisolone. No other pairwise comparisons demonstrated differences significant at the 0.05 level. However, the combination of BCNU plus high-dose methylprednisolone tended to be less effective than BCNU alone in patients with poor prognosis. This study indicates that BCNU and procarbazine are moderately useful agents in conjunction with radiotherapy for patients with malignant glioma. In addition, future protocols may allow use of corticosteroids in conventional dosages for treating cerebral edema and controlling symptoms; conclusions based on survival as the endpoint are unlikely to be affected by administering steroids at somewhat greater than the usual dose. More effective regimens for the treatment of malignant glioma should be sought. [Cancer Treat Rep 67:121–132, 1983]

The Brain Tumor Study Group (BTSG), a cooperative group of neurosurgeons, radiotherapists, neuroradiologists, neuropathologists, and oncologists working in conjunction with the National Cancer Institute, has recently reported the results of a randomized clinical trial (BTSG 72-01) comparing radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery (1). Included within the designation of malignant glioma are glioblastoma multiforme and malignant (anaplastic) astrocytoma. The results of BTSG 72-01, together with

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[2]This study was conducted by the Brain Tumor Study Group and was supported by contracts (N01-CM) from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: Bowman-Gray School of Medicine, Winston-Salem, NC (*E. Alexander, Jr*, N01-CM-67054); Duke University Medical Center, Durham, NC (*G. L. Odom*, N01-CM-67010); Indiana University School of Medicine, Bloomington (*J. Mealey, Jr*, N01-CM-67057); Sloan-Kettering Memorial Hospital, New York, NY (*W. R. Shapiro*, N01-CM-57019); New York University Medical Center, New York (*J. Ransohoff, II*, N01-CM-67059); Ohio State University Research Foundation, Columbus (*W. E. Hunt*, N01-CM-67060); St. Louis University School of Medicine, MO (*K. R. Smith, Jr*, N01-CM-57020); University of California Medical Center at San Francisco (*C. B. Wilson*, N01-CM-67055); University of California at Los Angeles (*U. Batzdorf*, N01-CM-53752); University of Kentucky Medical Center, Lexington (*W. H. Brooks*, N01-CM-67058); University of Tennessee Center for Health Sciences, Knoxville (*J. T. Robertson*, N01-CM-57021); Montefiore Hospital Association and the University of Pittsburgh, PA (*R. G. Selker*, N01-CM-67061); and Clinica Neurochirurgica Dell 'Universita di Pavia, Italy (*P. Paoletti*, N01-CM-67056). Also participating were the National Institute of Neurological and Communicative Disorders and Stroke (*M. D. Walker*) and the National Cancer Institute (*S. B. Green, D. P. Byar, D. A. Pistenmaa*, and *T. A. Strike*), National Institutes of Health, Bethesda, MD.

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those of a previous trial (BTSG 69-01) (2), demonstrated a significant increase in survival when surgery was followed by radiotherapy (total dose of 6000 rads) compared to surgery alone; they also suggested a modest further benefit from the addition of a nitrosourea to radiotherapy, which led to an increased percentage of "long-term" (18-month) survivors. In BTSG 72-01 the median survival of patients treated with radiotherapy plus nitrosourea was 10–12 months. Based on such studies our reference drug for chemotherapy of malignant glioma has been carmustine (BCNU).

Several approaches have been suggested in an attempt to improve on these results. Although corticosteroids are known to be important in the treatment of life-threatening cerebral edema and can produce dramatic improvement in neurologic status and symptoms (3), an antitumor effect of steroids leading to prolonged survival for patients with malignant glioma has not been demonstrated. However, experimental evidence has suggested possible oncolytic activity for corticosteroids (4–7). The BTSG therefore decided to investigate the effect of high-dose corticosteroids on survival in a prospective randomized clinical trial (BTSG 75-01), reported here, in order to determine (*a*) if an oncolytic effect for corticosteroids could be demonstrated and (*b*) the extent to which corticosteroids might confound survivorship observations in chemotherapeutic trials for malignant glioma. To increase the probability of observing an antitumor effect, doses of methylprednisolone approximately tenfold the conventional dosage used for the control of cerebral edema were utilized in an intermittent schedule.

Because of results in controlled phase II studies carried out by the BTSG, as well as reports in the literature, procarbazine was selected as an alternative chemotherapeutic agent for malignant glioma (8,9). Based on the above considerations, the present study was designed to compare four treatments: BCNU, high-dose methylprednisolone, procarbazine, and BCNU plus high-dose methylprednisolone.

After this study had begun, Lieberman et al (10) reported in 1977 the results of a small pilot study involving the use of high doses of methylprednisolone (200–2000 mg/day) in 11 patients with inoperable brain tumors. They observed neurologic improvement in eight patients. Serious adverse effects included sepsis in three patients (who also received chemotherapy) and myopathy in two patients, but the infections were treatable and no deaths could be attributed to steroids. Their report encouraged further investigation of high-dose corticosteroid.

Although the major purpose of clinical trials is the evaluation of treatment regimens, other useful information can be obtained from these studies. Prognostic factors such as patient and tumor characteristics, symptomatology, and neurologic examination are important objects of investigation, both for their own importance and as covariates for adjusted comparisons of treatment groups. An analysis of prognostic factors reported for BTSG 72-01 (1) has been extended in the present study.

METHODS

Patient Accrual

Thirteen institutions participated in the design and conduct of this trial. Patients were entered in the study by the principal investigators at each institution within 3 weeks of definitive surgical treatment for the primary tumor. Eligibility criteria included histologically demonstrated supratentorial malignant glioma, patient age of $\geq$ 15 years, and absence of major medical illness which could preclude treatment on any arm of this study. Eligible patients were not to have received any antineoplastic therapy prior to randomization, other than surgery (and conventional doses of corticosteroids within certain prescribed limits). All patients were informed of the nature of the study, the therapeutic methods that might be employed, the toxic effects that might reasonably be anticipated from such therapy, the randomization procedure, and the availability of continued care if they declined to enter or remain in the study. Written informed consent was obtained from all patients.

Patients were randomized to one of the four treatment groups by means of a telephone call to the BTSG Operations Office. Demographic, neurologic, and surgical information was collected for all patients, and a set of pathology slides from each patient's tumor was sent to the BTSG Operations Office for subsequent evaluation by the Neuropathology Review Committee, which made the final determination of eligibility on the basis of the histopathologic criteria given by Burger and Vogel (11).

Treatment

The prescribed radiotherapy for all patients was a total dose of 6000 rads in 30–35 fractions of 172–200 rads, 5 days/week over 6–7 weeks, delivered to the whole brain by parallel opposed ports with megavoltage equipment. The calibration of equipment at each institution and the dose delivered to each patient were verified by the Radiologic Physics Center, Houston, TX. The first course of chemotherapy was scheduled during the first few radiotherapy sessions. The protocol included provision for limited use of corticosteroids (dexamethasone or methylprednisolone) in conventional dosages during the perioperative period, including the time of radiotherapy, and also during subsequent courses of chemotherapy, for the control of life-threatening cerebral edema.

BCNU was administered iv at a dose of 80 mg/m²/day on 3 successive days every 8 weeks. For patients who developed thrombocytopenia (platelet count < 50,000/mm³), leukopenia (wbc count < 2500/mm³), or a decrease in hematocrit of > 5 percentage points, the subsequent dose was reduced to 60 mg/m²/day. Before the subsequent course could be administered, marrow recovery had to be sufficient to give a platelet count of at least 75,000/mm³, wbc count of at least 3000/mm³, and hematocrit of at least 30%. An additional dose reduction to 45 mg/m²/day was undertaken if the same indicators of toxicity were observed after treatment at a reduced dose.

Methylprednisolone at a total dose of 400 mg/m²/day was given orally in three divided doses (with the morning, noon, and evening meals) for 7 consecutive days without taper. After a 3-week interval, methylprednisolone administration was repeated and continued in this 1 week "on" and 3 weeks "off" cycle. Steroid-related toxic effects were cause for temporary discontinuation of therapy, and active gastrointestinal hemorrhage was considered an absolute contraindication for further methylprednisolone therapy.

Patients randomized to receive both BCNU and methylprednisolone were treated according to the schedules for each of the individual drugs as noted above. In the event that re-treatment was delayed because of toxicity, neither drug was given until recovery.

Procarbazine was given orally at a total dose of 150 mg/m²/day in three or four divided doses for 28 consecutive days every 8 weeks. Dosage was decreased to 100 or subsequently to 80 mg/m²/day for the same indications of toxicity used for BCNU, and with the same requirements for marrow recovery.

Before treatment, patients were evaluated by neurologic examination and were assigned a performance status rating according to the Karnofsky scale (12).[3] Patients also had a cbc and a platelet count before each course of treatment and at appropriate intervals during the course of treatment. Performance status was evaluated monthly. Neurodiagnostic tests, particularly computerized axial tomographic scans, were obtained when indicated.

Statistical Methods

We calculated the probability of survival for a specified length of time by the life-table method (13) and compared the survival of different treatment groups using the Mantel-Haenszel chi-square statistic (14), always with two-tailed tests.

We investigated the prognostic effect of individual covariates by examining death rates, defined as the number of deaths divided by the total number of patient-months of follow-up for all patients in a specific category, and we tested significance with a chi-square test for linear trend. These tests were intended only to serve as useful guides in the initial screening of variables. To study the prognostic importance of multiple variables acting simultaneously, we used regression models in which the risk of death is modeled as a function of the specified variables. Variables were eliminated from the regression model by a "step-down" procedure if they did not have a significant effect on survival given the other variables in the model. After applying such a procedure with the Cox proportional hazards model (15), we compared survival of the four treatment groups adjusted for the important prognostic factors retained in the model. Testing for significance of a treatment effect was based on the square of the standardized regression coefficient (the coefficient for the treatment variable in the Cox model divided by its standard error), which was compared with critical values for a chi-square with one degree of freedom. We identified groups of patients with differing risks of death predicted from their prognostic factors, using the approach described by Byar et al (16) with a Weibull regression model.

RESULTS

Between January 1976 and April 1978, 609 patients were randomized following surgery to one of the four treatment groups as follows: 147 to BCNU, 156 to methylprednisolone, 153 to procarbazine, and 153 to BCNU plus methylprednisolone. Patients on all four treatment arms were scheduled to receive radiotherapy.

We first performed analyses on the total randomized population. For subsequent analyses we excluded certain patients to produce a valid study group. The reasons for exclusion were as follows: 34 patients did not have malignant glioma on review of slides by the Neuropathology Review Committee; three patients did not have slides available for review; six patients were randomized in error (ie, they were ineligible); 32 patients did not receive the initial chemotherapy to which they were randomized; and seven patients did not receive any radiotherapy.

The valid study group on the basis of these criteria consisted of 527 patients, of whom 187 (35%) were female and 340 (65%) were male. Important characteristics of these patients have been tabulated by treatment group (table 1), and the extent of the treatment they

[3] 100, normal (no complaints, no evidence of disease); 90, able to carry on normal activity (minor symptoms); 80, normal activity with effort (some symptoms); 70, cares for self (unable to carry on normal activity); 60, requires occasional assistance (cares for most needs); 50, requires considerable assistance and frequent care; 40, disabled (requires special care and assistance); 30, severely disabled (hospitalized, death not imminent); 20, very sick (active supportive treatment needed); 10, moribund (fatal processes are rapidly progressing).

TABLE 1.—Characteristics of patients in the valid study group

Characteristic	Treatment group				All treatments
	BCNU	Methyl-prednisolone	Procarbazine	BCNU + methyl-prednisolone	
No. of patients	124	141	128	134	527
Median age at randomization (yrs)	57	56	56	56	56
Blood type (% of patients)*					
A	32	46	50	39	42
B	12	10	11	13	11
AB	5	4	2	5	4
O	51	40	37	43	43
Median duration of symptoms (mos)	2.0	2.3	1.9	2.1	2.1
Histopathologic category (% of patients)					
Glioblastoma multiforme	88.7	85.8	89.1	85.1	87.1
Malignant (anaplastic) astrocytoma	9.7	10.6	9.4	14.9	11.2
Other†	1.6	3.6	1.5	0	1.7
Median initial Karnofsky performance status	70	70	70	70	70
Abnormal consciousness level (% of patients)	21	17	16	17	18
Median pretreatment wbc count ($\times 10^3/mm^3$)	8.8	9.0	9.4	9.3	9.0
Median pretreatment platelet count ($\times 10^3/mm^3$)*	311	290	320	321	310

*Tabulations exclude patients whose values were not available (6 for blood type, 1 for platelet count).

†Includes the following categories: malignant ependymoma, astroblastoma, oligodendroblastoma, and other malignant glioma.

received is shown in table 2. Patient characteristics appear to be reasonably balanced among the four treatment groups. The data in table 2 suggest heterogeneity with respect to dose of radiotherapy received. However, this could be produced by variations in the number of patients surviving long enough to receive a full course, and in fact, the heterogeneity in mean radiotherapy dose disappears if calculations are limited to those patients surviving > 2 months. The group randomized to procarbazine received fewer courses of chemotherapy than the other groups, but the heterogeneity (in mean number of courses) among the four treatment groups was not statistically significant.

We first performed an unadjusted analysis of survival for the total randomized population, with survival measured from the time of randomization. Inspection of life-table survival probabilities (table 3) and the corresponding survival curves (fig 1) indicates that in general the four treatment groups had similar survival, except that from 12 months onward the group receiving only high-dose methylprednisolone had a lower percentage of survivors than each of the other three groups.

TABLE 2.—Extent of treatment received by patients in the valid study group

	Treatment group			
	BCNU	Methyl-prednisolone	Procarbazine	BCNU + methyl-prednisolone
Mean No. of courses of chemotherapy ± SE (either full or reduced dose)	4.5 ± 0.3	4.0 ± 0.3	3.7 ± 0.3	4.5 ± 0.3
% of patients receiving—				
≥ 2	80.7	82.2	77.4	78.4
≥ 3	62.2	61.6	51.6	64.2
≥ 4	54.1	46.0	41.4	53.8
≥ 5	42.8	34.7	28.9	47.1
Mean radiotherapy dose ± SE (rads)	5467 ± 119	5701 ± 79	5809 ± 57	5589 ± 96

TABLE 3.—Life-table survival probabilities by treatment group

Treatment group	No. of patients	Median survival (wks)	% surviving, ± SE			
			6 mos	12 mos	18 mos	24 mos
Total randomized population						
BCNU	147	50	68.3 ± 3.9	48.8 ± 4.2	26.5 ± 3.7	19.5 ± 3.3
Methylprednisolone	156	41	68.8 ± 3.7	33.7 ± 3.8	16.4 ± 3.1	8.0 ± 2.3
Procarbazine	153	43	71.3 ± 3.7	41.6 ± 4.0	28.6 ± 3.7	22.2 ± 3.5
BCNU + methylprednisolone	153	41	68.4 ± 3.8	40.5 ± 4.0	23.1 ± 3.5	18.0 ± 3.2
Valid study group						
BCNU	124	50	70.7 ± 4.1	48.5 ± 4.5	23.8 ± 3.9	15.6 ± 3.3
Methylprednisolone	141	40	68.6 ± 3.9	32.8 ± 4.0	15.1 ± 3.1	6.0 ± 2.1
Procarbazine	128	47	76.5 ± 3.8	44.0 ± 4.4	28.8 ± 4.0	22.8 ± 3.8
BCNU + methylprednisolone	134	41	69.4 ± 4.0	40.2 ± 4.2	22.9 ± 3.7	17.9 ± 3.4

Statistical tests (table 4) indicate that the survival experience of the groups receiving either procarbazine or BCNU alone was significantly better ($P < 0.05$) than that of the group receiving only methylprednisolone. The survival comparison between methylprednisolone alone versus its combination with BCNU was not statistically significant, nor were any of the other pairwise comparisons.

We next performed an unadjusted analysis of survival limited to the valid study group (table 3 and fig 2). The results are similar to those for the total randomized population, but in this analysis the better survival with BCNU plus methylprednisolone compared to methylprednisolone alone approached significance at the 0.05 level ($P = 0.062$, table 4). The results reinforce the impression that BCNU and procarbazine each improved survival compared with methylprednisolone alone.

We analyzed data from the valid study group to identify variables of prognostic importance to use in adjusted treatment comparisons. We considered variables related to patient characteristics, laboratory determinations, performance status, and other disease-related variables. Data were available both on symptoms reported by the patient and on findings of the neurologic examination at the time of randomization. These two sets of variables overlapped in many aspects (for example, presence of sensory deficit). In such situations we found, not unexpectedly, that the more objective (and better quantitated) variables obtained from the neurologic examination predicted survival more accurately than the more subjective variables reported by patients. In subsequent analyses we therefore used the variables based on the neurologic examination. Certain symptomologic variables that did not have a direct

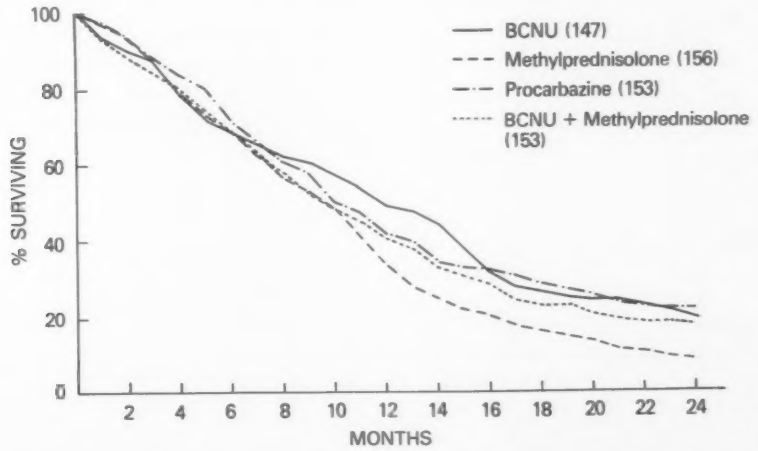

FIGURE 1.—Survival curves for each treatment group in the total randomized population. Nos. of patients are shown in parentheses.

TABLE 4.—Comparisons of survival of pairs among the 4 treatment groups

Treatment comparison	Total randomized population unadjusted (Mantel-Haenszel)*	Valid study group	
		Unadjusted (Mantel-Haenszel)*	Adjusted (Cox model)*
BCNU vs methylprednisolone	0.006	0.009	0.010
BCNU vs procarbazine	0.680	0.690	0.873
BCNU vs BCNU + methylprednisolone	0.252	0.621	0.123
Methylprednisolone vs procarbazine	0.017	0.003	0.014
Methylprednisolone vs BCNU + methylprednisolone	0.133	0.062	0.285
Procarbazine vs BCNU + methylprednisolone	0.418	0.354	0.164

*2-tailed P values.

counterpart in the neurologic examination, such as history of seizures, were retained in the investigation.

Altogether we identified 16 variables that significantly affected death rate when studied one at a time (table 5). Variables with values that change with time were assessed at or near the time of randomization (for example, at the postoperative pretreatment neurologic examination). Eight of these variables were eliminated from the Cox proportional hazards model without significant loss of ability of the model to predict survival. The eight remaining variables, each of which was important for predicting survival even in the presence of the other variables, were: patient age at randomization, histopathologic category, performance status, ABO blood type, pretreatment wbc count, pretreatment platelet count, duration of symptoms, and consciousness level as recorded on neurologic examination.

With these variables, we performed an adjusted analysis of survival of patients in the valid study group, using the Cox model with indicator variables to represent the treatment groups. In this and all subsequent multivariate analyses using these eight variables, we excluded seven patients for whom the value of one of the variables was not available, giving a total of 520 patients. The results of significance tests in the adjusted analysis (table 4) confirmed the general conclusions reached from the unadjusted analyses. The groups receiving either procarbazine or BCNU alone had significantly increased survival compared to the group receiving only methylprednisolone. The difference in survival between methylprednisolone alone and BCNU plus methylprednisolone was not statistically significant ($P > 0.20$).

With the set of eight prognostic variables used for adjustment, we categorized the patients into three risk groups based on 24-month survival predictions from the Weibull model (table 5, last column), as follows: Group 1, > 25% predicted survival (123 patients); Group 2,

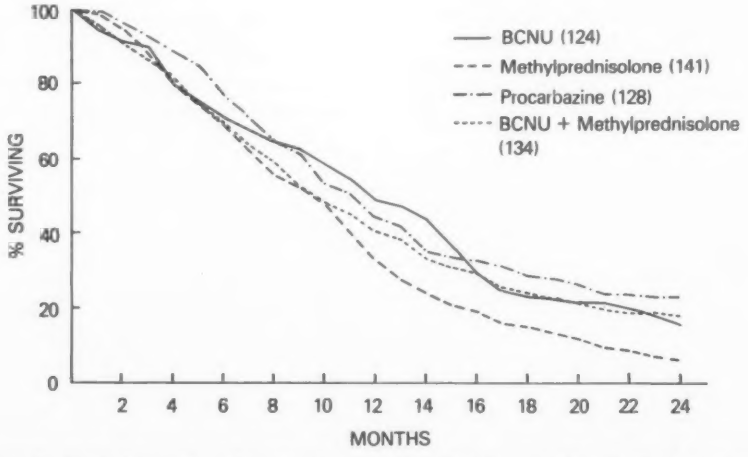

FIGURE 2.—Survival curves for each treatment group in the valid study group. Nos. of patients are shown in parentheses.

Table 5. Effect of Individual Prognostic Variables on Death Rate

VARIABLE	CATEGORY	NUMBER OF PATIENTS	NUMBER OF DEATHS	DEATH RATE*	P-VALUE	COEFFICIENT IN WEIBULL MODEL°
All patients		527	471	0.70		
Age at randomization (years)	<45	101	75	0.36	<0.00001	0.369
	45-54	127	110	0.63		
	55-64	192	183	0.89		
	≥65	107	103	1.27		
Blood type+	B,O	282	249	0.62	0.0008	0.217
	A,AB	239	217	0.84		
History of arteriosclerosis	No	495	439	0.68	0.0001	
	Yes	32	32	1.39		
History of Cancer	No	500	444	0.69	0.0066	
	Yes	27	27	1.17		
History of Seizures	No	362	335	0.84	<0.00001	
	Yes	165	136	0.50		
Duration of Symptoms (months)	≤6	432	399	0.81	<0.00001	-0.443
	>6	95	72	0.41		
Resection Type	Biopsy only	24	23	0.96	0.0021	
	Subtotal	414	376	0.73		
	Total	63	52	0.66		
	Total + lobectomy	26	20	0.39		
Histopathologic category	Other malignant glioma	68	51	0.43	0.0001	0.397
	Glioblastoma multiforme	459	420	0.76		
Performance status (Karnofsky scale)	10-40	52	52	1.31	<0.00001	-0.230
	50-60	153	144	0.96		
	70-80	216	189	0.66		
	90-100	106	86	0.44		
Pre-treatment white-cell count (thousands/mm^3)	5.5-8.5	188	163	0.59	0.0024	0.243
	other	339	308	0.79		
Pre-treatment platelet count+ (thousands/mm^3)	≤200	42	40	0.99	0.0014	-0.166
	201-300	207	191	0.79		
	>300	277	240	0.63		
Neurological examination:						
Consciousness level	Normal	434	380	0.64	<0.00001	0.414
	Abnormal	93	91	1.28		
Motor Disorder	None	184	154	0.54	<0.00001	
	Slight-moderate	255	231	0.75		
	Marked-severe	88	86	1.12		
Sensory Disorder	None	392	344	0.65	0.0003	
	Slight-moderate	106	99	0.87		
	Marked-severe	29	28	1.14		
Speech Disorder	None-slight	401	347	0.63	<0.00001	
	Moderate-severe	126	124	1.04		
Cranial nerve abnormality (other than III,IV,VI)	None-slight	462	410	0.68	0.0113	
	Moderate-severe	65	61	0.96		

*Death rates are expressed as the number of deaths per 10 patient-months.

+Tabulations exclude patients whose values were not available (6 for blood type, 1 for platelet count).

°Coefficients are given for the eight variables retained in the multivariate analysis after a "step-down" procedure. Variables were coded by assigning each category a consecutive integer starting with zero. Weibull intercept parameter =-4.207; shape parameter =1.401.

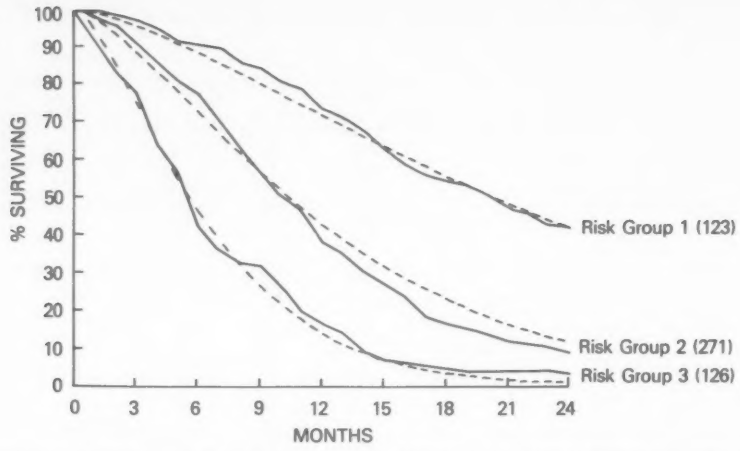

FIGURE 3.—Survival curves for each risk group as defined in the text. Solid lines represent observed life-table survival rates. Dashed lines represent predicted survival rates from the Weibull model. Nos. of patients are shown in parentheses.

2.5%–25% (271 patients); and Group 3, < 2.5% (126 patients). A graph of the observed life-table survival curves for these three groups, along with the predicted curves from the model (fig 3), indicates that the model provides a good fit to the data and demonstrates the potential value of these prognostic variables in identifying patients who differ markedly in expected survival.

While recognizing the potential pitfalls in making historical comparisons, we decided to compare the survival curves of selected treatment groups in the present study (BTSG 75-01) with their counterparts in BTSG 72-01. These two studies had the same eligibility criteria, with the exception that a few patients < 15 years of age were admitted to BTSG 72-01 (there were three such patients in the valid study group). The same centralized neuropathology review was performed in the two studies. One arm of the randomization, BCNU plus radiotherapy, was the same in both studies, with the same dose levels and similar guidelines for dose modification. Furthermore, if it is accepted that the present study provides evidence that high-dose methylprednisolone does not affect survival, then the methylprednisolone group in BTSG 75-01 (which also received radiotherapy)

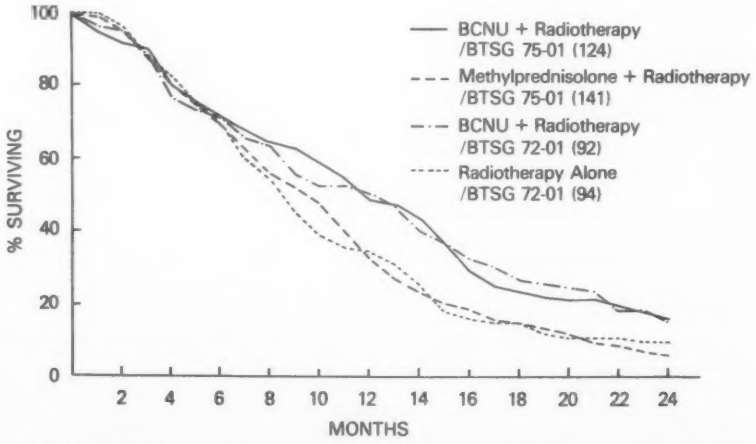

FIGURE 4.—Comparison of selected survival curves for valid study group patients from BTSG studies 72-01 and 75-01. Nos. of patients are shown in parentheses.

may be considered comparable to the group in BTSG 72-01 that received radiotherapy alone. With this hypothesis in mind, we plotted the corresponding survival curves from the valid study groups (fig 4). The curves for the two groups that received BCNU plus radiotherapy are practically superimposed. In addition, the curves for the two groups that did not receive BCNU are also quite similar to each other, and both separate from the BCNU pair at approximately 12 months. Thus, if methylprednisolone is not considered to be antitumor chemotherapy, the results suggest a modest improvement in survival after the first year when a chemotherapeutic agent, such as BCNU, was added to radiotherapy and surgery. Using the Cox model, we calculated adjusted statistics to compare the two BCNU groups and to compare the two non-BCNU groups, adjusting on four important prognostic factors for which data were available in both studies (table 6). Neither of the statistics was significant ($P > 0.30$ for each), consistent with an absence of a difference across studies.

Lastly, we tabulated toxicity reported for patients in the valid study group for the present study. Hematologic toxicity was defined as a wbc count $< 3500/mm^3$, platelet count $< 100,000/mm^3$, or hematocrit $< 30\%$; the data have been tabulated by treatment group and treatment course (table 7). In addition, qualitative information was collected on the incidence of a variety of adverse medical sequelae during the time these patients were receiving their assigned chemotherapy, and a statistical test for heterogeneity was performed to detect any difference in incidence among the four treatment groups (table 8). These data are presented as general indications of toxicity and could possibly represent an underestimate of overall incidence, in that we cannot be certain that every occurrence of these conditions was reported. Furthermore, only those sequelae occurring during chemotherapy courses were tabulated; whether or not conditions occurring subsequently should have been included, had the information been available, depends on the definition of drug-related toxicity. Dermatologic or allergic reactions, and nausea, vomiting, or diarrhea showed significant heterogeneity among treatment groups; a significantly greater number of patients with these conditions were reported in the procarbazine group. Infection showed significant heterogeneity; a significantly greater number of patients with infections were reported in the group receiving BCNU plus high-dose methylprednisolone. Skeletomuscular complications also showed significant heterogeneity, occurring in 6.5% of patients receiving high-dose methylprednisolone (with or without BCNU) compared to 1.6% of patients in the other two groups. In addition, definite gastrointestinal hemorrhage was reported in ten patients, eight of whom were in one of the groups receiving high-dose methylprednisolone, but this difference was not statistically significant. The principal investigators had been requested to alert the BTSG Operations Office of other adverse effects of high-dose steroids. Many of the patients experienced varying degrees of depression, of brief duration, when high-dose methylprednisolone was discontinued at the end of each

TABLE 6.—Distribution of prognostic variables used for adjusted comparisons of selected treatment groups (valid study groups) from BTSG studies 72-01 and 75-01

Variable and category	% of patients in treatment group			
	BCNU + radiotherapy 75-01 (124 patients)	Methylprednisolone + radiotherapy 75-01 (141 patients)	BCNU + radiotherapy 72-01 (92 patients)	Radiotherapy alone 72-01 (94 patients)
Age at randomization (yrs)				
< 45	19.4	21.3	27.2	9.6
45–54	21.8	24.1	26.1	34.0
55–64	33.1	31.9	26.1	33.0
≥ 65	25.8	22.7	20.7	23.4
Histopathologic category				
Glioblastoma multiforme	88.7	85.8	81.5	87.2
Other malignant glioma	11.3	14.2	18.5	12.8
Karnofsky performance status				
10–40	11.3	10.6	21.7	19.1
50–60	25.8	28.4	28.3	36.2
70–80	42.7	42.6	25.0	13.8
90–100	20.2	18.4	25.0	30.9
Duration of symptoms (mos)				
≤ 6	79.8	81.6	84.8	79.8
> 6	20.2	18.4	15.2	20.2

TABLE 7.—Hematologic toxicity by treatment for patients in the valid study group

Course	Treatment	Wbc count* ($\times 10^3$/mm^3) Pretreatment	Wbc count* ($\times 10^3$/mm^3) Nadir	Platelet count* ($\times 10^3$/mm^3) Pretreatment	Platelet count* ($\times 10^3$/mm^3) Nadir	Hematocrit* (%) Pretreatment	Hematocrit* (%) Nadir
1	BCNU	9.7 (124)	2.4 (56)	338 (124)	55 (43)	37 (124)	27 (15)
	Methylprednisolone	9.8 (141)	3.2 (1)	317 (141)	90 (1)	37 (141)	28 (4)
	Procarbazine	10.0 (128)	2.0 (64)	348 (127)	70 (34)	37 (128)	28 (13)
	BCNU + methylprednisolone	10.0 (134)	2.3 (44)	348 (134)	65 (37)	37 (133)	26 (20)
2	BCNU	6.3 (100)	2.5 (38)	271 (100)	60 (30)	39 (100)	27 (8)
	Methylprednisolone	7.1 (115)	2.5 (1)	294 (110)	64 (1)	39 (115)	28 (1)
	Procarbazine	5.9 (99)	2.5 (36)	263 (99)	78 (15)	38 (99)	26 (5)
	BCNU + methylprednisolone	6.5 (104)	2.7 (25)	316 (104)	75 (24)	37 (104)	26 (10)
3	BCNU	5.3 (77)	2.5 (35)	249 (77)	62 (28)	39 (77)	26 (2)
	Methylprednisolone	7.2 (83)	2.9 (5)	285 (83)	64 (2)	39 (83)	26 (1)
	Procarbazine	4.6 (66)	2.6 (29)	212 (66)	73 (15)	38 (66)	27 (4)
	BCNU + methylprednisolone	6.0 (86)	2.7 (23)	281 (86)	67 (32)	38 (86)	25 (8)
4	BCNU	4.9 (67)	2.7 (25)	239 (67)	70 (19)	38 (67)	27 (4)
	Methylprednisolone	6.8 (60)	2.8 (2)	298 (60)	— (0)	40 (60)	25 (1)
	Procarbazine	4.6 (53)	2.6 (30)	193 (53)	63 (15)	37 (53)	28 (5)
	BCNU + methylprednisolone	5.8 (71)	2.6 (18)	228 (71)	60 (19)	38 (71)	25 (4)

*Values = mean. No. of patients contributing to each value is shown in parentheses. Mean nadir values are based on those patients with wbc count $< 3.5 \times 10^3$/mm^3, platelet count $< 100 \times 10^3$/mm^3, or hematocrit < 30%.

cycle. A few instances of side effects such as early cushingoid symptoms or early cataract were reported, but otherwise there were no serious complications.

DISCUSSION

The results of this prospective randomized study indicate that procarbazine and BCNU each provided a significantly increased survival for patients with malignant glioma when compared with methylprednisolone, even though the latter agent had been given in high doses to enhance possible oncolytic effect. However, there was not a significant difference in survival between the groups of patients receiving either procarbazine or BCNU alone. Although historical comparisons must be interpreted with caution, the comparison of methylprednisolone plus radiotherapy in the present study with radiotherapy alone in the previous study (BTSG 72-01) suggests that high-dose corticosteroids following surgery and radiotherapy did not increase survival compared to surgery and radiotherapy alone.

TABLE 8.—Incidence of adverse sequelae during chemotherapy*

Sequel	% of patients in treatment group: BCNU	Methyl-prednisolone	Procarbazine	BCNU + methyl-prednisolone	*P* value for heterogeneity among treatment groups
Thrombophlebitis	11.3	6.4	7.0	10.4	0.399
Pulmonary complication (emboli, fibrosis, etc)	16.1	9.9	8.6	13.4	0.235
Change in chest film or purified protein derivative test	3.2	7.1	0.8	4.5	0.064
Dermatologic or allergic reaction	4.0	8.5	25.0	14.2	< 0.001
Infection	17.7	19.1	23.4	34.3	0.006
Severe nausea, vomiting, or diarrhea	4.8	2.1	12.5	5.2	0.003
Diabetes (uncontrolled)	0.0	3.5	0.8	0.0	0.016
Skeletomuscular complications	1.6	7.8	1.6	5.2	0.026

*For each treatment group, values = % of patients in valid study group for whom indicated sequelae were reported at any time during chemotherapy.

These results, however, do not diminish the importance of corticosteroids in lower doses for treating cerebral edema and controlling symptoms. In addition, these results support and strengthen the conclusions of previous studies that chemotherapeutic agents such as BCNU or procarbazine can modestly increase the percentage of "long-term" survivors. In practice the choice between these two agents for any specific patient may depend on considerations of differential toxicity. The fact that patients in the procarbazine group received fewer courses of chemotherapy may result from their increased incidence of gastrointestinal and allergic reactions. However, the survival of the procarbazine and BCNU groups was similar in both the unadjusted analysis and the analysis adjusted on initial prognostic factors.

The evaluation of the combination of BCNU plus high-dose methylprednisolone is less certain. The overall conclusion is that the survival of this group of patients did not differ significantly from any of the other groups. The survival curves suggested that this combination was better than methylprednisolone alone but not quite as good as BCNU alone. In the absence of statistical significance, it is important not to overinterpret such results since they may be due to chance. However, the data suggest the possibility that adding high-dose methylprednisolone to BCNU had an adverse effect, at least for some patients. First, the overall incidence of infections was higher with the combination; however, data on type or severity of infection were not available for analysis. Second, we compared treatments separately in each of the three risk groups described above and found that the position of the curve for BCNU plus methylprednisolone, relative to the other three groups, dropped as the predicted survival worsened. This analysis suggests that, if the combined regimen was inferior to BCNU alone, this effect tended to occur in patients with poor prognosis.

Whether or not this suggestion (ie, that adding high-dose corticosteroids to chemotherapy may be harmful for some patients) is in fact true, our results indicate that using steroids in amounts considerably higher than those required for treating cerebral edema and controlling symptoms does not increase survival. Thus, with this endpoint we do not find evidence for an antitumor effect of high-dose corticosteroids. The patients in this study were allowed to receive corticosteroids in conventional dosages as needed, and in the absence of a control group the study was not designed to provide new information on the effectiveness of such conventional therapy. However, the results do indicate that future protocols can allow the use of corticosteroids in conventional dosages, as required by a patient's condition, without concern that administering steroids at somewhat greater than the usual dose will confound the interpretation of other treatment comparisons, providing that survival is used as the endpoint.

Any analyses performed on patient groups other than the total randomized population may be biased due to exclusion of patients. For this reason we first presented an analysis which included all randomized patients, before proceeding to an analysis of the valid study group adjusted for prognostic factors. No important differences in conclusions were found.

As discussed previously in the report of BTSG 72-01 (1), it is unlikely that multiple studies would select the identical set of significant prognostic variables, because of the effects of chance and because of correlations among various prognostic factors. The present analysis confirms that patient age, histopathologic category, performance status at time of randomization, and symptom duration are important prognostic variables, even when used together. Additional information available in the present study showed the prognostic importance of certain findings on neurologic examination and of certain laboratory determinations. Of particular interest is the finding that patients with blood group A (whether type A or AB) had decreased survival compared to those with type B or O. In an unpublished analysis of a concurrent BTSG phase II study we found similar results for the effect of blood type, significant at $P < 0.01$ after adjustment for age, performance status, and histopathologic category. Preliminary analysis of two subsequent studies showed a smaller effect which was not statistically significant. There is experimental evidence that some tumor cells possess antigens that are related to blood group A antigens (17). If malignant gliomas have A-like tumor antigens, then patients with blood type B or O who have anti-A antibodies may mount a more effective immunologic response against tumor cells. This speculation must await further study.

While this study has indicated that procarbazine is as useful an agent as BCNU for the chemotherapy of malignant glioma, given in combination with radiotherapy, the search for more successful regimens must be continued. Ongoing randomized clinical trials by the BTSG are designed to investigate different approaches to radiotherapy and chemotherapy for this disease.

REFERENCES

1. Walker MD, Green SB, Byar DP, et al. Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl J Med 303:1323–1329, 1980.
2. Walker MD, Alexander E, Jr, Hunt WE, et al. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas: a cooperative clinical trial. J Neurosurg 49:333–343, 1978.
3. Gutin PH. Corticosteroid therapy in patients with cerebral tumors: benefits, mechanisms, problems, practicalities. Semin Oncol 2:49–56, 1975.

4. Wright RL, Shaumba B, and Keller J. The effect of glucocorticosteroids on growth and metabolism of experimental glial tumors. J Neurosurg 30: 140–145, 1969.
5. Gurcay O, Wilson C, Barker M, et al. Corticosteroid effect on transplantable rat glioma. Arch Neurol 24:266–269, 1971.
6. Mealey J, Jr, Chen TT, and Schanz GP. Effects of dexamethasone and methylprednisolone on cell cultures of human glioblastomas. J Neurosurg 34:324–334, 1971.
7. Shapiro WR, and Posner JB. Corticosteroid hormones. Effects in an experimental brain tumor. Arch Neurol 30:217–221, 1974.
8. Kumar ARV, Renaudin J, Wilson CB, et al. Procarbazine hydrochloride in the treatment of brain tumors—phase II study. J Neurosurg 40:365–371, 1974.
9. Walker MD. Chemotherapy: adjuvant to surgery and radiation therapy. Semin Oncol 2:69–72, 1975.
10. Lieberman A, LeBrun Y, Glass P, et al. Use of high dose corticosteroids in patients with inoperable brain tumours. J Neurol Neurosurg Psychiatry 40:678–682, 1977.
11. Burger PC, and Vogel FS. Surgical pathology of the nervous system and its coverings. New York, John Wiley, 1976, pp 189–373.
12. Karnofsky DA, Abelmann WH, Craver LF, et al. The use of the nitrogen mustards in the palliative treatment of carcinoma: with particular reference to bronchogenic carcinoma. Cancer 1:634–656, 1948.
13. Cutler SJ, and Ederer F. Maximum utilization of the life table method in analyzing survival. J Chronic Dis 8:699–712, 1958.
14. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 50:163–170, 1966.
15. Cox DR. Regression models and life tables. J R Stat Soc [B] 34:187–202, 1972.
16. Byar DP, Green SB, Dor P, et al. A prognostic index for thyroid carcinoma: a study of the E.O.R.T.C. Thyroid Cancer Cooperative Group. Eur J Cancer 15:1033–1041, 1979.
17. Bloom ET, Fahey JL, Peterson IA, et al. Anti-tumor activity in human serum: antibodies detecting blood-group-A-like antigen on the surface of tumor cells in culture. Int J Cancer 12:21–31, 1973.

Clinical Evaluation of Long-Term, Continuous-Infusion Doxorubicin[1,2,3]

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We have performed preclinical and clinical pharmacology evaluations of continuous-infusion doxorubicin (ADR), utilizing a totally implantable subcutaneous drug delivery system (Infusaid, Metal Bellows Corp, Norwood, MA). The preclinical studies in dogs established the compatibility of ADR solutions in contact with the titanium and stainless steel components of the Infusaid pump, the long-term stability of ADR at physiologic temperatures, and the reliability of pump function and flow under the conditions of clinical use. In preclinical studies in dogs, myelosuppression was found to be the dose-limiting toxicity at a dosage of 0.15 mg/kg/day and was a rapidly reversible phenomenon followed by rebound thrombocytosis and leukocytosis. In the phase I investigation, 14 patients with cancer resistant to standard therapies were treated. The Infusaid pump was surgically implanted in the infraclavicular fossa or epigastrium; the Silastic delivery catheter was placed in the subclavian vein in 13 patients and the external carotid artery in one patient. During the study, 174 pump refills with ADR were completed without refill-related extravasation of drug or infection at the injection site. Based on our animal toxicity data, the starting dose was 1 mg/m^2/day with escalation by 1-mg/m^2/day increments every 3 weeks. Dose-limiting toxic effects were myelosuppression, conjunctivitis, and oral mucositis. Alopecia was also observed. Technical complications were observed in nine patients and are described. Potentially serious intravascular thromboses related to the subclavian catheter were detected in three patients. Endomyocardial biopsies were performed in two patients who had achieved a cumulative ADR dose of 682 and 652 mg/m^2; cardiotoxicity scores of 1 and 2A (Stanford Scoring System), respectively, were recorded. Urine and plasma pharmacology studies using high-performance liquid chromatography were performed in all patients to monitor ADR, adriamycinol, and aglycone metabolites. Of the total anthracycline infused over a 24-hour period, 5.4% was recovered in the urine (range, 0.7%–16%). Plasma levels were undetectable in all but two patients in whom drug levels were transiently measurable. The results support the use of the Infusaid drug delivery system as a feasible method of long-term infusion of ADR. Based on our toxicity data, a starting dosage for continuous infusion ADR at 4.0 mg/m^2/day is proposed. [Cancer Treat Rep 67:133–142, 1983]

Doxorubicin (ADR) is an anthracycline anticancer antibiotic of value in the treatment of a wide variety of solid tumors, lymphomas, and leukemias (2). The utility of ADR is limited by its acute and long-term cumulative toxicity. Acute toxicity is characterized by dose-limiting myelosuppression, stomatitis, alopecia, and nausea and vomiting. In addition, when administered on standard dose schedules (iv bolus every 3 weeks), ADR car-

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ries a risk of symptomatic cardiac toxicity at a cumulative dose > 450 mg/m² (3). Attempts to enhance the therapeutic index might reasonably begin by seeking methods to reduce these toxic sequelae.

The long-term continuous infusion of chemotherapeutic agents offers one approach to altering the therapeutic index. Several studies have been performed in which the schedule of drug administration was a randomized variable. These indicate that more frequent administration of chemotherapeutic agents is superior to more intensive intermittent therapy. In such a comparison of combination chemotherapy in patients with metastatic breast cancer (4), a significantly higher response rate was observed with more frequent administration of therapy.

Recent data also suggest that weekly administration of ADR permits delivery of greater cumulative doses with less incidence of acute toxicity and delayed cardiac toxicity and with no evident loss of antitumor activity. ADR treatment by continuous infusion over brief periods has had similar results (5–10).

Continuous-infusion chemotherapy has been limited by the lack of convenient methods for long-term drug infusion. Previous techniques have utilized external portable pumps or long-term transcutaneous catheters. These methods have the attendant hazards of prolonged hospitalization, infection, thrombosis, and potentially serious paravenous drug extravasation. Recent technologic advances in self-contained, implantable infusion devices make prolonged drug infusion possible. The Infusaid implantable, subcutaneous drug delivery system (Metal Bellows Corp, Norwood, MA) allows prolonged continuous infusion of heparin and anticancer agents (11–15). Outpatient drug administration utilizing the system may continue indefinitely, perhaps obviating many complications of percutaneous catheter placement and external pumping devices. The present report describes the preclinical development, phase I trial, and clinical pharmacology studies of long-term, continuous-infusion ADR by the Infusaid pump.

MATERIALS AND METHODS

Infusaid Pump

Drug delivery was accomplished by the Infusaid pump, a titanium alloy disk 8.6 cm in diameter, which contains a titanium bellows infusate reservoir of 50-ml capacity (fig 1). The bellows is powered by a volatile liquid fluorocarbon, which is in equilibrium with its vapor phase at 37°C; constant pressure is generated across the infusate reservoir relatively independent of volume. The infusate is delivered at a nearly constant rate through a stainless steel capillary flow restrictor and a radiopaque Silastic elastomer catheter, which may be implanted in the desired vessel. The infusate reservoir is entered with a needle through the skin and a self-sealing silicone rubber septum. The force required to expand and refill the reservoir recharges the fluorocarbon pressure source.

Doxorubicin-Infusaid Compatibility Studies

ADR in sterile saline solution (1.0 and 2.0 mg/ml) was continuously maintained at 37°C in contact with the Infusaid bellows to determine the long-term stability of such formulations. ADR and its decomposition products were monitored by high-performance liquid chromatography (HPLC) with flow fluorescence detection as previously described (16); for direct monitoring of drug for chemical stability, this technique permits detection of fluorescent breakdown products in the picomolar range. By measuring both the area of the ADR signal and the appearance and quantitation of the fluorescence signals for ≤ 14 days, it was found that ADR in solution decomposed < 4% over 7 days, with decomposition becoming pronounced after Day 9. In parallel studies monitoring weekly refill cycles for ≤ 16 weeks, flow characteristics were not altered over the 7–9-day refill cycle, nor did insoluble material affect the cellulose acetate exit filter.

Cuttings of titanium metal rings of which the bellows device is constructed were also exposed to ADR solutions at 37°C. No effect of the metal alloy on the ADR formulation could be detected, although the metal was clearly stained and permanently discolored by the drug solution; prolonged contact of the bellows metal with ADR solutions did not alter the metallurgic properties of the titanium alloy (E. Tucker, Metal Bellows Corp, personal communication).

Two standard model pumps were filled with ADR solution and were permitted to operate at 37°C for 10 weeks; each pump was refilled with fresh ADR solution weekly. The total eluate was collected and serially measured for the presence of dissolved iron during passage through the stainless steel capillary flow restrictor. Iron was measured by colorimetric analysis (American Monitor Corp; sensitivity, 1 part per million) and by atomic absorption spectroscopy (subnanogram sensitivity) at the conclusion of 10 weeks. No increase in the iron content of the eluate compared to the intrinsic iron content of the ADR formulation was detected.

No difference in the flow rate of the pump was detected in a comparison of eluates of saline with an ADR/lactose excipient/saline formulation.

Preclinical Dog Toxicologic and Pharmacologic Studies

A series of animal studies were undertaken in an attempt to characterize the maximal tolerated dose of

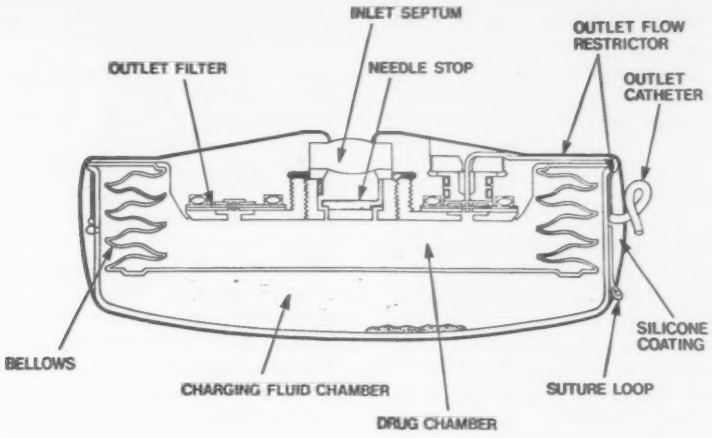

FIGURE 1.—Schematic diagram of model 100 pump. Drug chamber is a titanium bellows enclosed by a fluorocarbon-filled fluid chamber. Volatile liquid fluorocarbon is in equilibrium with its vapor phase at 37°C. When drug chamber is full and bellows expanded, vapor pressure of fluorocarbon is about 300 mm Hg greater than atmospheric pressure. Pressure is transmitted across drug chamber bellows and slowly delivers infusate through filter into outlet tubing, stainless steel flow restrictor, and catheter. Flow rate of pump is set by outlet flow restrictor and is mathematically represented by Pouiselle's equation: $Q = \pi D^4 \Delta P / 128 \mu L$, where Q = flow (milliliters per second), D = tubing inside diameter (centimeters), μ = fluid viscosity (poise), ΔP = pressure (dynes/square centimeter), and L = tubing length (centimeters). Drug chamber is filled by penetrating inlet septum percutaneously with Huber-tip needle and forcing drug under pressure into expandable bellows. Capacity of pump drug chamber is 50 ml.

continuous-infusion ADR, to correlate plasma levels and metabolism of the drug with observed toxicity, and to evaluate the reliability of pump function after implantation. An Infusaid pump was implanted in a beagle dog. The pump delivered ADR in doses ranging from 0.08 to 0.18 mg/kg/day over a 12-week period, which correspond to 1.6–3.6 mg/m²/day in man (17). (To convert doses for dogs, in milligrams per kilogram, to doses for man, in milligrams per square meter, a factor of 20 is used.) Liver and renal function tests and wbc and platelet counts were performed every 4 days, and ADR levels were monitored weekly over the course of the study.

In a second investigation, a starting dosage of 0.225 mg/kg/day (equivalent to 4.5 mg/m²/day in man) was utilized. ADR plasma levels and wbc and platelet counts were monitored daily to record the production of and recovery from acute hematologic toxicity.

Clinical Studies

Surgical Implantation

Prior to implantation, the pump reservoir was filled with sterile water, brought to physiologic temperature in a water bath, and permitted to express all air from the flow restrictor line and Silastic catheter. After flow was established, the pump was surgically implanted in a subcutaneous pocket at the infraclavicular fossa or epigastrium. The position of the pump was fixed by suturing loops of Dacron around the perimeter of the device to the underlying fascia. The attached Silastic catheter was implanted in the subclavian vein by means of a Cook (Bloomington, IN) catheter sheath devised for catheter or pacemaker wire placement. In one patient, however, the catheter was placed by an arteriotomy in the left external carotid artery for perfusion of an orbital osteosarcoma. The overlying wound was closed primarily without drains.

One week following surgery, the pump was filled with ADR; it was then refilled with fresh drug weekly in the outpatient clinic.

Clinical Eligibility Criteria

Patients with histologically confirmed carcinoma or sarcoma who had failed or never received therapy of proven clinical benefit were eligible for the study. Other criteria for patients were performance status ≤ 2 on the Eastern Cooperative Oncology Group (ECOG) scale

and full recovery from toxic effects associated with prior therapy. History was recorded; physical examination, chest X-ray, electrocardiogram, liver function tests, and cbc were performed; and serum creatinine and BUN were determined. Written informed consent was obtained from all patients.

Protocol for ADR Infusion

The toxic dose low of ADR in the dog was 0.2 mg/kg/day. We have determined that a safe dosage of ADR via continuous infusion in the dog was 0.1 mg/kg/day. Based on surface area equivalents as previously discussed, 0.1 mg/kg/day in the dog equals 0.05 mg/kg/day in man. Since the conversion factor in man is 37, the appropriate equivalent dosage is 2 mg/m^2/day. It was decided that a safe starting dosage in patients should be 50% of the safe dosage in experimental animals (50% of 0.1 mg/kg/day in the beagle dog or 1 mg/m^2/day in man). Calculated starting dosages were escalated by 1-mg/m^2 increments above the initial 1-mg/m^2/day dosage. At least two patients were treated at each of the calculated dosage levels before escalation took place. However, variation in pump flow rates at initiation of treatment introduced a dosage error requiring calculation of a true starting dosage for each patient, as noted in table 1.

Patients returned weekly to the outpatient clinic for pump refilling with fresh ADR. In the absence of toxicity at the preceding dosage level, escalations occurred every 3 weeks in 1-mg/m^2/day increments. Preclinical animal studies suggested that dose-limiting toxicity would be myelosuppression. Hence, if a patient's wbc count was $> 4000/mm^3$ and platelet count was $> 100,000/mm^3$, ADR infusion at a given dosage level continued for 3 weeks. At the end of the 3-week period, if wbc and platelet counts remained at or above this level, the dosage of ADR was increased by 1 mg/m^2/day for the next 3-week period. If the wbc count was 3000–4000/mm^3 and the platelet count was 90,000–100,000/mm^3 at the time of scheduled dose escalation, the preceding dose was maintained. If any administered dose resulted in wbc count $\leq 3000/mm^3$ or platelet count $\leq 90,000/mm^3$, ADR was discontinued for 1 week. When wbc and platelet counts returned to levels $> 3000/mm^3$ or 90,000/mm^3, respectively, ADR infusion was resumed at a dosage 0.5 mg/m^2/day less than the previous dose. Otherwise, if recovery of blood cell counts was not observed in 1 week, treatment was not resumed until recovery occurred.

Dose adjustments based on other observed toxic effects (oral mucositis or conjunctivitis) were performed by means of a similar schema. Nonhematologic toxicity was graded utilizing the ECOG scale. Appearance of oral mucositis of grade 2 or greater led to treatment interruption of ≥ 1 week until toxicity resolved. Treatment was resumed at a calculated dosage of 0.5 mg/m^2/day less than the previous dosage. A lesser grade of toxicity led to continuance of treatment until resolution or progression of toxicity permitted dosage escalation or necessitated treatment interruption.

Patients evaluable for antitumor response were judged on the basis of accepted criteria.

Cardiac Toxicity Monitoring

Endomyocardial biopsies were performed in two patients who received cumulative doses of ADR > 500 mg/m^2, when a therapeutic decision regarding continuance of the drug was necessary. Biopsy tissue was obtained transvenously from the septal wall of the right ventricle under fluoroscopic guidance, using the technique of Mason (18). Biopsy tissue was immediately fixed in 2% buffered glutaraldehyde. The fixed specimen was sent to Dr. Margaret Billingham (Stanford University Medical Center, CA) for Epon embedment, sectioning, and electron microscopic examination for cardiotoxicity scoring.

Pharmacology Studies

At weekly outpatient visits, the patients provided urine specimens obtained during the preceding 24 hours, and a blood specimen was collected in heparin at the time of pump refilling. Heparinized plasma and urine samples were adjusted to pH 8.5, extracted with chloroform and methanol, dried under nitrogen, analyzed immediately or frozen, stored at –80°C, and protected from light. Aliquots of extracts were reconstituted in methanol and monitored according to previously published HPLC techniques for the analysis of levels of ADR, adriamycinol, and other metabolites (16). This methodology has a lower limit of sensitivity for precise quantitation of 5 ng/ml in plasma and urine; levels in the range of 0.8–5.0 ng/ml are detectable but not accurately quantifiable. In addition, the unused ADR recovered during the pump-refilling procedure was routinely analyzed by HPLC for stability and breakdown products.

RESULTS

Animal Toxicology

The beagle dog was treated with continuous-infusion ADR for 11 weeks at dosages ranging from 0.09 to 0.18 mg/kg/day (fig 2). Myelosuppression (wbc count, 4800/mm^3; normal 6000–17,000) was first noted during Week 9 of treatment, 7 days after elevation of treatment dosage to 0.15 mg/kg/day (3 mg/m^2/day in man). When the dosage was increased to 0.18 mg/kg/day at Week 10, a

TABLE 1.—Toxic effects of ADR*

Patient No.	Dosage† (mg/m²/day)	Toxic effect	Cumulative dosage (mg/m²)	Duration of infusion (wks)
1	SD: 2.0	None	(14)	(1)
	MD: 2.0			
2	SD: 1.0	None	(22)	(3.5)
	MD: 2.0			
3	SD: 1.5	None	(112)	(7)
	MD: 4.0			
4	SD: 4.0	None	(295)	(9)
	MD: 8.0			
5	SD: 5.0	None	(254)	(10)
	MD: 5.0			
6	SD: 4.0	None	(677)	(21)
	MD: 7.5			
7	SD: 3.0	None	(956)	(28)
	MD: 9.0			
8	SD: 5.0			
	TD: 4.5	Mucositis	68	(2)
	MD: 5.0		(120)	(4)
9	SD: 5.0			
	TD: 6.5	Leukopenia, mucositis	113	3
	TD: 7.0	Thrombocytopenia	187	4
	MD: 8.5		(246)	(8)
10	SD: 4.0			
	TD: 6.5	Mucositis	161	5
	MD: 6.5		(269)	(10)
11	SD: 6.0			
	TD: 5.0	Leukopenia	113	3
	TD: 5.0	Leukopenia	123	4
	TD: 4.5	Leukopenia	218	6
	MD: 6.0		(357)	(12)
12	SD: 5.0			
	TD: 5.0	Mucositis	67	2
	TD: 3.5	Mucositis	119	4
	TD: 3.5	Leukopenia	143	5
	TD: 2.5	Mucositis	204	8
	TD: 3.0	Mucositis	263	11
	MD: 5.0		(307)	(14)
13	SD: 1.0			
	TD: 3.5	Leukopenia	159	13
	MD: 3.5		(249)	(22)
14	SD: 2.0			
	TD: 7.0	Conjunctivitis	744	24
	MD: 7.0		(744)	(24)

*SD = starting dosage, MD = maximum dosage level achieved, TD = dosage level at toxicity. Values in parentheses are totals.
†True dosage level delivered; pump flow rate variability may have introduced an error in delivering ADR dosage at calculated dosage escalation or de-escalation based on observed toxicity.

wbc count nadir of 2700/mm³ was reached on the 7th day at this dosage. The study was concluded 1 week later with development of a wound infection overlying the pump implantation site. Pharmacologic monitoring of plasma ADR by HPLC detected levels at 5 weeks of treatment (10 ng/ml at a dosage of 0.1 mg/kg/day). Dosage increments were accompanied by increasing plasma levels of ADR, reaching a maximum level of 29 ng/ml at a dosage of 0.18 mg/kg/day at Week 10 of treatment. Platelet counts were not seriously suppressed during the 11-week study.

In a second beagle dog study, continuous-infusion ADR was started at a dosage of 0.225 mg/kg/day (4.5 mg/m²/day in man) (fig 3). One week later, dosage was increased to 0.25 mg/kg/day. Severe leukopenia (200 cells/mm³) and thrombocytopenia (15,000 cells/mm³) occurred on Day 7 at this dosage. Drug infusion was discontinued at that time; within 3 days there was prompt recovery of wbc and platelet counts. Rebound leukocytosis (14,000 cells/mm³) developed 7 days after conclusion of treatment. Plasma levels of ADR during infusion reached 55–65 ng/ml.

No other toxic effects were observed in the animal studies.

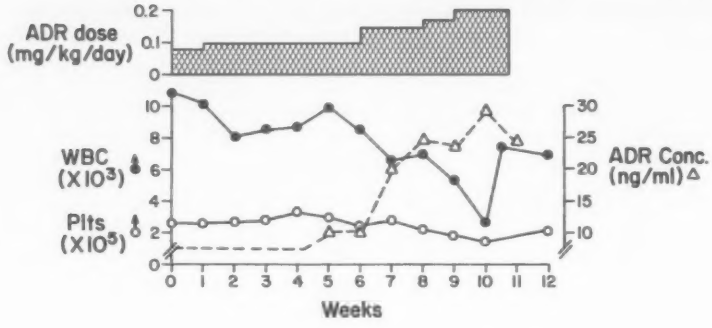

FIGURE 2.—● = wbc count (cells × 10^3/mm^3); ○ = platelet count (cells × 10^5/mm^3). Modest myelotoxicity was induced in the beagle dog during 11-wk infusion of ADR. Plasma ADR levels (Δ) were detectable during Week 5 of treatment and increased with dosage increments of infused ADR. Infusion was terminated when wound infection developed at pump implantation site.

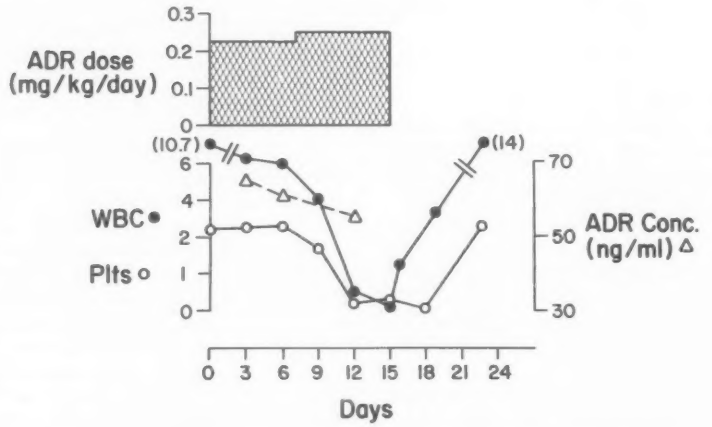

FIGURE 3.—● = wbc count (cells × 10^3/mm^3); ○ = platelet count (cells × 10^5/mm^3). Acute myelotoxicity was induced in the dog by continuous-infusion ADR at a dose of 0.225 mg/kg/day for 2 wks. Leukopenia and thrombocytopenia were profound at 2 wks. Prompt wbc and platelet count recovery was observed at conclusion of treatment, accompanied by rebound elevation above pretreatment values. Δ = plasma ADR levels during drug infusion.

Clinical Studies

Fourteen patients, eight males and six females, were entered into the study. Patient characteristics are presented in table 2. Of the patients treated with continuous-infusion ADR, four had received prior therapy with ADR. One hundred seventy-four transcutaneous pump refills with ADR were completed without complication.

One major concern at the start of the study was the potential for skin toxicity if ADR inadvertently extravasated during the pump filling or emptying. Seromas containing ADR developed in two patients; one patient experienced pump migration and catheter extraction and one experienced pump leakage. At the time of pump removal from one patient, a portion of subcutaneous tissue was surgically excised and shown to contain ADR. The wounds healed in all cases without evidence of skin toxicity (19).

TABLE 2.—Patient characteristics

Characteristic	
Total No. of patients	14
Male/female	8/6
Age in yrs	
Median	48
Range	22–65
No. of patients with—	
Performance status (ECOG)	
0	10
1–2	4
Previous therapy	
Chemotherapy	5
Radiation/chemotherapy	6
None	3
No. of tumor types treated	
Renal adenocarcinoma	5
Soft tissue sarcoma	4
Breast	3
Colorectal	2
No. of pump fills	174
No. of pump implantations	18

The goal of this study was to establish a dosage at which ADR-induced toxicity would appear, would become dose-limiting, and could be made tolerable for the duration of continuous infusion. The dose-limiting toxicity observed at each dosage-week unit of treatment is presented in tables 1 and 3; toxic effects include oral mucositis, conjunctivitis, leukopenia, and thrombocytopenia. Toxicity requiring treatment interruption and dosage adjustment was first observed at an ADR dosage of 2.5 mg/m²/day. Further toxicity was observed with increasing dosage, yet three patients tolerated doses > 7.0 mg/m²/day without toxic effects (maximum, 9 mg/m²/day). Only five episodes of toxicity occurred among 96 weekly courses of treatment at doses ≤ 4.0 mg/m²/day (average, one toxic episode per 19.2 weekly courses), while ten episodes occurred among 78 courses at ≥ 4.5 mg/m²/day (average, one episode per 7.8 weekly courses). In 174 weeks of ADR infusion, 28 dosage escalations were achieved (mean, two escalations per patient). The small number of weeks of treatment at dosages > 6.0 mg/m²/day precludes analysis of the incidence and severity of toxicity at these levels.

Both myelosuppression and mucositis developed after 14–21 days of treatment at a given toxic dosage and, because of the defined dosage adjustment scheme, always led to dosage adjustment. When therapy was interrupted, recovery occurred within 1 week in eight episodes of toxicity (two mucositis and six myelosuppression) but was prolonged > 1 week in the remaining seven episodes (five mucositis, one conjunctivitis, and one myelosuppression).

In table 4, the number of patients who eventually developed dose-limiting toxic effects is viewed as a function of starting dosage; three of nine patients begun at a dosage ≤ 4 mg/m²/day developed toxic sequelae requiring dosage adjustment, whereas four of five patients begun at a dosage ≥ 5.0 mg/m²/day ultimately developed dose-limiting toxic effects. The data in table 1 suggest that dose-limiting toxicity was not a product of the cumulative ADR dosage administered but simply an event more likely to occur with higher starting dosage. All oral mucositis leading to treatment interruption was grade 2 or greater on the ECOG toxicity scale.

TABLE 3.—Dose-limiting toxicity

Dosage* (mg/m²/day)	No. of patients	Treatment duration (wks)	No. of episodes of toxicity requiring dosage adjustment			
			Mucositis	Leukopenia	Thrombocytopenia	Other
1.0	3	11				
1.5	4	9				
2.0	4	8				
2.5	6	15	1			
3.0	8	19	1			
3.5	6	12	1	2		
4.0	10	22				
4.5	7	16	1	1		
5.0	9	18	1	2		
5.5	3	7				
6.0	4	12				
6.5	4	7	2	1		
7.0	4	9			1	1†
7.5	2	2				
8.0	2	4				
8.5	2	2				
9.0	1	1				

*Corrected for variations in pump flow rate and rounded to nearest 0.5 mg/m²/day.
†Conjunctivitis.

TABLE 4.—Toxic effects of ADR at starting dosage

Starting dosage ($mg/m^2/day$)*	No. of patients	
	Total	Requiring dosage adjustment for toxicity
1.0	2	1
1.5	1	0
2.0	2	1
3.0	1	0
4.0	3	1
5.0	4	3
6.0	1	1

*Corrected for variations in pump flow rate and rounded to nearest 0.5 $mg/m^2/day$.

No episode of oral mucositis resulted in hospitalization, parenteral fluid maintenance, or complete discontinuance of oral intake. In the six episodes of leukopenia observed during treatment and requiring interruption of infusion, the median wbc count nadir was 2200/mm^3 (range, 1400–3000). The single platelet count nadir was 61,000/mm^3.

Other toxic effects included partial alopecia in four patients and total alopecia in four. All patients experienced mild fatigue and mild loss of appetite; three had nausea or vomiting, which was controlled with medication and was not dose-limiting.

Technical Complications

Technical complications unrelated to ADR infusion developed in nine patients (64%). Catheter migration and "pull-out" occurred in three patients, and two had subcutaneous ADR extravasation. The pump was promptly removed, and the wounds were irrigated, closed, and permitted to heal by primary intention. Healing occurred without ulceration or tissue necrosis. Catheter kinking/occlusion in one patient appeared to be related to excessive mobility of the pump within the implantation site. Catheter lumen clots were an additional complication in three patients. Because heparin and ADR form a visible precipitate when combined in solution, heparin was not added to the infusate. Nonheparinized solution was the only substance in contact with the catheter lumen. In a fourth patient, pump malfunction with ADR leakage into the subcutaneous tissue occurred, which required pump removal. Also, the large size of the pump interfered with wound healing in one patient; the pump was removed and replaced with a smaller model. In summary, technical complications in five patients necessitated pump removal; the pump was reimplanted in four of these patients.

Autopsy in one patient who died of cancer revealed an organized thrombus at the tip of the catheter; the thrombus was adherent to the endothelium of the superior vena cava and right atrium. In addition, a recent pulmonary embolus was found in this patient. A second patient developed a subacute superior vena cava syndrome. A superior vena cavagram showed nearly complete obstruction of the left subclavian and innominate veins along the course of the Silastic catheter, with extensive collateral blood flow through the neck and chest. At the time of death from progressive cancer, autopsy confirmed extensive organized thrombosis of the left subclavian vein. In a third patient, a radionuclide superior vena cavagram performed prospectively showed evidence of incomplete obstruction to caval blood flow. A subsequent contrast study confirmed incomplete obstruction of the subclavian vein and superior vena cava, presumably by thrombus propagation along the length of the catheter. This patient remained without symptoms referable to the thrombosis until death from progressive cancer.

The reliability of the flow rate of the Infusaid pump was variable. In 81 of 174 weeks of treatment, the flow rate of the pump varied by 0.5 ml/day more or less than the flow rate reported by the manufacturer. This variation in flow rate tended to be lower than the expected rate in most patients, suggesting that the viscosity of the concentrated ADR solution slowed pump flow.

Therapeutic Response

All 14 patients were evaluable for response to therapy. One patient with metastatic breast carcinoma experienced a response to ADR lasting 3 months, which was characterized by a $> 30\%$ reduction in palpable liver size in the midclavicular line and by a $> 50\%$ decrease in plasma CEA.

Cardiac Toxicity Studies

Among the 14 patients, the cumulative dosage of ADR infused ranged from 14 to 956 mg/m^2 by the conclusion of the study. The median cumulative dosage was 254 mg/m^2; the mean was 330 mg/m^2. Two patients underwent endomyocardial biopsy at cumulative ADR dosages of 682 and 652 mg/m^2. Biopsy histopathology scores (Stanford Scoring System) were 1 and 2A, respectively.

If ADR had been infused on conventional 3-week scheduling, expected scores at these cumulative doses would have been ≥ 2 (20,21). Both patients ultimately received dosages of 956 and 744 mg/m^2, respectively, with no evidence of cardiac dysfunction by clinical examination, electrocardiography, or radionuclide ventriculography.

Pharmacologic Monitoring

Urine and plasma pharmacology specimens were obtained in all patients. Reported values were the mean of two or three determinations performed per specimen. Detectable plasma levels of ADR were found transiently in two patients: 10 ng of ADR/ml in the plasma of a patient treated at a dosage of 2.0 mg/m^2/day for 1 week and 13 ng of ADR/ml and 12 ng of adriamycinol/ml in the plasma of a patient treated at 6 mg/m^2/day. All other determinations yielded no detectable plasma levels in any other patients treated at all other dosage levels. Mean total urinary excretion of infused anthracyline in all patients was 5.4% in a 24-hour period (range, 0.7%–16.4%); 3.7% was unaltered, and 1.7% was adriamycinol.

DISCUSSION

The technique for prolonged continuous infusion of anticancer agents has been limited by the lack of a reliable and practical pumping mechanism that was sufficiently portable, did not cross a skin-vascular barrier, and did not require hospitalization for the duration of drug infusion. The Infusaid implantable pump has resolved many of the technical dilemmas. It has been employed clinically over the past 3$^1/_2$ years for the continuous iv infusion of heparin, FUdR (6,9), and methotrexate (8). This study confirms that long-term infusion of ADR is a feasible method of outpatient drug administration. However, 64% of the patients had a technical complication leading to infusion interruption or pump removal. Although no patient experienced infection of the implantation site or drug extravasation related to transcutaneous pump filling, subcutaneous ADR extravasation did occur in three patients.

Subclavian implantation of the Infusaid device and administration of nonheparinized solutions carried a 21% risk of potentially serious thrombotic complications in this study, a rate exceeding that of any other published experience with the device. However, the mechanical complications (catheter withdrawal and kinking) appear quite amenable to resolution by simple alterations in techniques of pump and catheter anchoring. Hence, an issue of patient safety must be addressed only when use of nonheparinized solution in the Infusaid device is considered for iv delivery.

The therapeutic index of ADR and its analogs is severely restricted by acute reversible toxicity and cumulative cardiac toxicity when the drug is administered by conventional techniques and schedules. Recent evidence indicates that alterations of the treatment schedule may reduce both the acute and long-term toxicity without jeopardizing therapeutic efficacy. Reports of weekly infusions of ADR by Weiss and Manthel (9), Weiss et al (10), and Chlebowski et al (5) concur that acute toxicity is substantially ameliorated by more frequent lower doses. Similarly, Gercovich et al (6) and Legha et al (7,8) studied continuous-infusion schedules of brief duration (10 hours to 4 days). They reported that these infusion modifications produced very little acute gastrointestinal and hematologic toxicity. In addition, all studies noted a lower frequency of clinical cardiac toxicity than expected in patients receiving cumulative ADR dosages > 600 mg/m^2. Legha et al showed endomyocardial biopsy and histopathologic data supporting a lessening of cardiac toxicity with the continuous-infusion schemes. In studies in which plasma levels of ADR were monitored, the authors felt that a reduction in peak plasma levels might be responsible for the reduction of schedule dependence in acute and long-term toxicity.

No other published studies have examined the plasma pharmacokinetics of ADR administration at dosages similar to ours. However, Legha et al (8) reported the results of determination of plasma ADR levels during infusion at a dosage of 60 mg/m^2. As the duration of infusion of this dosage was extended from 5 minutes to 96 hours, peak plasma levels dropped tenfold (1.31–0.097 μg/ml, measured as total plasma fluorescence). In our study, it is surprising that plasma levels of ADR remained undetectable at the dosage of 9.0 mg/m^2/day, which approaches that in the study by Legha et al, approximately 15.0 mg/m^2/day, particularly in light of the sensitivity of our HPLC assay. Reconciliation of these differences will require continued pharmacokinetic monitoring of plasma ADR levels, perhaps at higher dosages than we used.

We have shown that continuous-infusion ADR in cumulative doses surpassing those attained by standard infusion methods is practicable and has tolerable noncardiac toxicity. Dose-limiting toxic effects were oral mucositis, conjunctivitis, or myelosuppression. On the basis of our data, we recommend a starting dosage of 4.0 mg/m^2/day of ADR by continuous infusion, with dosage increments every 3rd week as tolerated.

We intend to pursue continuous-infusion ADR therapy in the treatment of patients with soft tissue sarcoma and breast carcinoma previously untreated with ADR. Additional endomyocardial biopsies will be performed to provide data regarding cardiac toxicity at high cumulative doses of ADR. Insofar as prolonged infusion of ADR is possible with the Infusaid pump, issues deserving major attention include documentation of re-

duced cardiac toxicity and establishment of objective response data in ADR-sensitive cancers.

REFERENCES

1. Garnick MB, Weiss GR, Steele GD, et al. Phase I trial of long term continuous Adriamycin administration. Proc Am Assoc Cancer Res and ASCO 22:359, 1981.
2. Blum RH, and Carter SK. Adriamycin: a new anticancer drug with significant clinical activity. Ann Intern Med 80:249–259, 1974.
3. Von Hoff DD, Layard MW, Basa P, et al. Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med 91:710–717, 1979.
4. Hoogstraten B, George SL, Samal B, et al. Combination chemotherapy and Adriamycin in patients with advanced breast cancer. Cancer 38:13–20, 1976.
5. Chlebowski RT, Paroly WS, Pugh RP, et al. Adriamycin given as a weekly schedule without a loading course: clinically effective with reduced incidence of cardiotoxicity. Cancer Treat Rep 64:47–51, 1980.
6. Gercovich FG, Praga C, Beretta G, et al. Ten-hour continuous infusion of Adriamycin. Proc Am Assoc Cancer Res and ASCO 20:372, 1979.
7. Legha SS, Benjamin RS, Yap HY, et al. Augmentation of Adriamycin's therapeutic index by prolonged continuous iv infusion for advanced breast cancer. Proc Am Assoc Cancer Res and ASCO 20:261, 1979.
8. Legha SS, Benjamin RS, Mackay B, et al. Reduction of doxorubicin cardiotoxicity by prolonged continuous intravenous infusion. Ann Intern Med 96:133–139, 1982.
9. Weiss AJ, and Manthel RW. Experience with the use of Adriamycin in combination with other anticancer agents using a weekly schedule with particular reference to lack of cardiac toxicity. Cancer 40:2046–2052, 1977.
10. Weiss AJ, Metter GE, Fletcher WS, et al. Studies on Adriamycin using a weekly regimen demonstrating its clinical effectiveness and lack of cardiac toxicity. Cancer Treat Rep 60:813–822, 1976.
11. Blackshear PJ, Dormon FD, Blackshear PJ, Jr, et al. The design and initial testing of an implantable infusion pump. Surg Gynecol Obstet 134:51–56, 1972.
12. Blackshear PJ, Rhode TD, Varco RL, et al. One year of continuous heparinization in the dog using a totally implantable infusion pump. Surg Gynecol Obstet 141:176–186, 1975.
13. Buchwald H, Grage TB, Vassilopoulos PP, et al. Intraarterial infusion chemotherapy for hepatic carcinoma using a totally implantable infusion pump. Cancer 45:866–869, 1980.
14. Dakhil S, Ensminger W, Kindt G, et al. Implanted system for intraventricular drug infusion in central nervous system tumors. Cancer Treat Rep 65:401–411, 1981.
15. Ensminger W, Niederhuber J, Dakhil S, et al. Totally implanted drug delivery system for hepatic arterial chemotherapy. Cancer Treat Rep 65:393–400, 1981.
16. Israel M, Pegg WH, Wilkinson PM, et al. Liquid chromatographic analysis of Adriamycin and metabolites in biological fluids. J Liq Chromatogr 1:795–809, 1978.
17. Freireich EJ, Gehan EA, Rall DP, et al. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother Rep 50:219–244, 1966.
18. Mason JW. Techniques for right and left ventricular endomyocardial biopsy. Am J Cardiol 41:887–892, 1978.
19. Garnick MB, Israel M, Khetarpal V, et al. Persistence of anthracycline levels following dermal and subcutaneous Adriamycin extravasation. Proc Am Assoc Cancer Res and ASCO, 22:173, 1981.
20. Billingham ME, and Bristow MR. Morphologic changes of anthracycline-induced heart disease. *In* Proceedings of the Conference on Long Term Normal Tissue Effects of Cancer Treatment. Bethesda, MD, Apr 13–15, 1981.

High-Dose Cytoreductive Therapy With Autologous Bone Marrow Transplantation in Advanced Malignancies[1,2]

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Twenty-one patients with advanced malignancies received high-dose chemotherapy and/or radiotherapy followed by autologous bone marrow infusion. Eighteen patients (85.7%) had fever ≥ 100°F for a median of 6 days; 14 of these patients required broad-spectrum antibiotics for a median of 13 days. Nineteen patients (90.5%) had a granulocyte count < 500/mm³ for a median of 11 days. Thrombocytopenia (platelet count < 50,000/mm³) was observed in 18 patients (85.7%) for a median of 14 days. Mucositis and diarrhea were not common, occurring in six (28.6%) and seven (33.3%) patients, respectively. Of the 21 patients studied, 16 were evaluable for tumor response; there were four complete responses and four partial responses, and two patients who showed no change for variable times. Two patients have unmaintained remissions for > 2 years. Our response rate (complete plus partial) is 50%. Our study shows that high-dose cytoreductive therapy can be given with moderate toxicity when combined with autologous bone marrow infusion. Because responses in this group of patients are generally of short duration, we believe that patients with advanced malignancies who have had less exposure to therapy or who have a high likelihood of disease recurrence should be considered for high-dose cytoreductive therapy with autologous transplantation. [Cancer Treat Rep 67:143–148, 1983]

It has been hypothesized that total tumor cell kill is a function of the dose of an effective drug (1). In general, when high doses of a drug can be given, a substantial increase in the response rate occurs (2). The major dose-limiting factor of most chemotherapeutic agents is the myelotoxicity which results in increased risk of infection (3). Therefore, modulation of myelotoxicity should allow an increased dose of an effective drug to be given, resulting in a greater antitumor response.

Studies conducted in the 1960s and 1970s showed that marrow recovery occurred following lethal doses of irradiation and/or chemotherapy when animals were protected by infusions of autologous bone marrow (4–7). Several studies in man, using a variety of cytotoxic regimens, have shown a lessening or shorter duration of myelotoxicity with adjunctive autologous bone marrow transplantation (8–17).

We administered several chemotherapeutic regimens at higher than normal doses followed by infusion of cryopreserved autologous bone marrow cells in patients who: (*a*) had widely metastatic disease; (*b*) had failed to respond to extensive conventional therapy; or (*c*) had tumors generally nonresponsive to conventional therapy.

METHODS

Twenty-one patients at Roswell Park Memorial Institute underwent autologous bone marrow transplantation preceded by high-dose chemotherapy. The patients

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were between 10 and 60 years of age. All autotransplant patients met the following criteria prior to therapy: (*a*) the CNS was functionally normal (normal mentation, memory, and ability to give informed consent); (*b*) there were no signs of infection at the initiation of the autotransplant and chemotherapy regimen; (*c*) at the time of aspiration, bone marrow cellularity was ≥ 2+ and the marrow differential cell count was within normal limits; (*d*) cardiac ejection fraction was > 50% (clinical heart failure requiring cardiac glycosides excluded patients from this study); (*e*) there was no clinical evidence of compromised pulmonary function; (*f*) bilirubin was ≤ 1.2 mg/dl; (*g*) creatinine was ≤ 2.5 mg/dl; and (*h*) hemoglobin was ≥ 10 g/dl.

Preparation of Bone Marrow

The process of bone marrow aspiration and storage has been previously described in detail (10). Briefly, while patients were under general or spinal anesthesia 1000–1500 ml of marrow was aspirated from multiple sites on the posterior iliac crest. During the operative procedure, all patients received two units of previously irradiated (1500 R) packed rbcs together with normal saline solution to equal the volume removed. In the operating room, the marrow was filtered through screens with 0.22-μm pores (Millipore Corp, Bedford, MA). The suspension was placed in 600-ml Fenwall bags and centrifuged at 4400 × *g* for 10 minutes, after which the supernatant was discarded and the remaining cells were placed in 50-ml polystyrene conical tubes and centrifuged at 2000 × *g* for 15 minutes. The buffy coat was withdrawn with a plastic Pasteur pipette and diluted to 100,000 cells/mm^3 with autologous plasma. The cryoprotective solution (consisting of one part autologous plasma, one part DMSO, and two parts tissue culture medium [Medium 199, GIBCO, Grand Island, NY] containing preservative-free heparin [10 units/100 ml]) was added to this suspension in a ratio of one part marrow material to one part cryoprotective solution at 4°C. Aliquots of marrow in freezing solution were placed in 50-ml blood-freezing bags (Delmed, Canton, MA) and cryopreserved at 1°C/minute to –40°C and then at 10°C/minute to –80°C using a programmed freezer (Cryomed, Mt. Clemens, MI). The marrow was then stored in either the fluid or vapor phase of a liquid nitrogen freezer until subsequent use.

Preparation of Patient

All patients had an indwelling catheter (Hickman or CVP) placed before initiation of chemotherapy, which was maintained and left in place until marrow recovery was evident. Furthermore, prior to the initiation of chemotherapy, patients were placed in single-patient rooms. During the initial phase of these studies, all people entering the patient's room wore masks, and prior to touching the patient, washed their hands with soap and water. However, other than handwashing, these precautions were discontinued after experience demonstrated the relatively low degree of problems with infection.

Some of the regimens for autologous bone marrow transplantation were as follows:

Melanoma
Carmustine, 400 mg/m^2 on Days 1–3
Transplant on Day 7
Renal cell carcinoma
Vinblastine, 30 mg/m^2 on Day 1
Carmustine, 400 mg/m^2 on Days 4–6
Transplant on Day 10
Testicular carcinoma, lymphoma (Hodgkin's and non-Hodgkin's), and acute leukemia
Cyclophosphamide, 50 mg/kg on Days 1–3
Carmustine, 400 mg/m^2 on Days 4–6
Transplant on Day 10

One patient with acute myeloblastic leukemia received piperazinedione at a dose of 50 mg on 2 consecutive days, followed by 5 days of rest, total-body irradiation at 800 rads, and then reinfusion with autologous marrow. One patient with diffuse mixed lymphocytic lymphoma received radiation therapy to the total abdomen, mantle, and Waldeyer's ring. All patients other than those with malignant melanoma or renal cell carcinoma had had extensive therapy prior to transplantation, and all patients had widely metastatic disease. All therapies produced moderate to severe bone marrow toxicity.

At the time of marrow reinfusion, the marrow was brought to the patient's bedside on dry ice. The patient was premedicated with 100 mg of hydrocortisone and 2500 units of heparin. Each 50-ml aliquot of marrow was thawed by repeated brief immersion into a 56°C water bath, manipulating the bag until no ice crystals were palpable or observable. While still cold, the marrow was aspirated into a 50-ml syringe and injected immediately into the patient. After each aliquot, patients were observed regarding symptoms, blood pressure, and pulse. The procedure was repeated at no less than 10-minute intervals with each 50-ml bag until all the marrow was reinfused. Following reinfusion, urine output was maintained at > 100 ml/hour for the ensuing 24 hours and was spot-checked for free hemoglobin using Hema-combistix (Ames, Elkhart, IN).

All patients received trimethoprim-sulfamethoxazole, two tablets twice a day, as prophylaxis. All blood products were irradiated with 1500 R prior to administration and all patients received at least 1500 calories/day either by regular diet, nutritional supplements, and/or parenteral nutrition.

Response was defined as: *complete (CR)*—the disappearance of all signs of the disease; *partial (PR)*—a > 50% regression of disease as determined by the prod-

uct of the cross-sectional diameters of the index tumor, without evidence of progression of other tumors or the appearance of new lesions; *minor*—a regression of disease that cannot qualify as PR but is > 25% as measured by the cross-sectional diameters of the index tumor; *no change*—no change or a < 25% change (either increase or decrease) in tumor during the period of observation; and *disease progression*—a ≥ 25% increase in tumor size as measured by the product of the cross-sectional diameters.

RESULTS

Twenty-one patients at Roswell Park Memorial Institute underwent autologous bone marrow transplantation preceded by high-dose chemotherapy. The patients were between 10 and 60 years of age. Generally, the patients tolerated the chemotherapy, bone marrow aspiration, and reinfusion well. At the time of reinfusion, three patients developed transient hypotension which normalized within 10 minutes of the infusion. No patient had a syncopal episode related to the reinfusion. Four patients also described chest pain while the marrow was being reinfused, again spontaneously normalizing within 10 minutes. Transient episodes of hypothermia and chills were seen in four patients, and nausea and vomiting occurred in two patients. Four patients developed transient hemoglobinuria after the infusion, which was probably secondary to the infusion of lysed rbcs, but the urine cleared in all patients after 24 hours of iv hydration.

Table 1 shows the events following bone marrow reinfusion. Nineteen patients (90.5%) had a granulocyte count < 500/mm³ for a median of 11 days (range, 3–32). Thrombocytopenia (platelet count < 50,000/mm³) was observed in 18 patients (85.7%) for a median of 14 days (range, 1–34). The patient with non-Hodgkin's lymphoma who was treated with radiation therapy did not have a granulocyte count < 500/mm³ or a platelet count < 50,000/mm³. Two patients with renal cell carcinoma had a granulocyte count < 500/mm³, while the platelet count stayed > 50,000/mm³. The patient with seminoma had a reduction of the platelet count to < 50,000/mm³, while the granulocyte count remained > 500/mm³. The last three patients received only chemotherapy as preparation for autotransplant. It is impossible to ascertain whether bone marrow recovery would have occurred anyway or whether the bone marrow transplant "rescued" these patients prior to hematologic toxic effects. The latter explanation is favored because in at least three of the four patients there was a significant effect on at least one cell line.

TABLE 1.—Marrow and nonmarrow toxicity

Side effect	No. of patients (%)	Median duration in days (range)
Fever (100°F)	18 (85.7)	10 (1–35)
Neutropenia (granulocyte count < 500/mm³)	19 (90.5)	11 (3–32)
Thrombocytopenia (platelet count < 50,000/mm³)	18 (85.7)	14 (1–34)
Mucositis	6 (28.6)	10 (5–38)
Diarrhea	7 (33.3)	4 (1–23)
Use of antibiotics	14 (66.6)	13 (4–36)

One patient with renal cell carcinoma developed the syndrome of inappropriate antidiuretic hormone secretion that led to severe hyponatremia and seizures. The hyponatremia was corrected and subsequently the patient had no recurrence of this electrolyte imbalance. The etiology of syndrome of inappropriate antidiuretic hormone in this case was probably due to the use of vinblastine and/or cyclophosphamide.

Table 2 shows patient characteristics, previous therapy, response, duration of response, and survival. Patients 1 and 2 had a CR, and they continue with no therapy and no evidence of disease for 2+ years. Patient 10 had a PR to the therapy, although of short duration. He underwent a second autotransplant and although he tolerated it well and his bone marrow reconstituted, there was disease progression and he died. Patient 14 had a CR, but died postoperatively 21 days following bone marrow reinfusion after an attempt was made to surgically drain liver abscesses occurring in the sites of previous tumor. Intraoperatively, it was found that there was no evidence of tumor, but previous tumor sites were necrosed.

Of a total of 21 patients studied, all were included in determining side effects, but five were not evaluable for tumor response because of early death (three from sepsis and two from disease progression in the CNS). Of the remaining 16 patients, four had CR, four had PR, and two showed no change for variable times. Our response rate (CR and PR) is 50%.

DISCUSSION

This study demonstrates that high-dose cytoreductive therapy, which is normally extremely toxic to the bone marrow, can be given safely when followed by reinfusion of cryopreserved autologous bone marrow cells. A total of eight patients (CR and PR) with widely metastatic disease unresponsive to conventional therapy or known to have a very low order of response to conventional therapy derived benefit from high-dose therapy plus autologous bone marrow autotransplantation. Two patients remain in unmaintained remission for > 2 years.

TABLE 2.—Patient characteristics

Patient No.	Diagnosis	Sex/age (yrs)	Recovery of marrow	Previous therapy*	Response†	Duration of response (days)	Cause of early death	Survival (days)
1	Burkitt's lymphoma	M/10	Yes	V, C, ADR, P, MTX	CR	730+		730+
2	Diffuse mixed lymphocytic lymphoma	F/50	Yes	ADR, C, V, P	CR	790+		790+
3	Acute myeloblastic leukemia	M/59	No	ADR, ARA-C	NE		Sepsis	7
4	Chronic myelocytic leukemia (blastic transformation)	M/37	No	BUS, 6-MP	NE		Sepsis	20
5	Seminoma	M/42	Yes	*Cis*-Pt, VBL, Bleo	NR			40
6	Acute myelocytic leukemia	M/53	Yes	ADR, ARA-C	NR			79
7	Embryonal cell carcinoma	M/41	Yes	VBL, *Cis*-Pt, Bleo, VP-16	NE		Sepsis	20
8	Melanoma	F/51	Yes	None	NC	120		382+
9	Melanoma	F/38	Yes	None	NR			30
10	Hodgkin's disease	M/36	Yes	MOPP, ABVD, and RT	PR	30		60
11	Hodgkin's disease	M/19	Yes	MOPP, ABVD	PR	30		60
12	Renal cell carcinoma	F/55	Yes	None	PR	120		280
13	Acute lymphoblastic leukemia	M/24	Yes	V, L-ASP, MTX, ARA-C, DNR, P, 6-MP, CNS irradiation	CR	67		106
14	Squamous cell carcinoma metastasizing to liver	M/56	Yes	None	CR	21		21
15	Melanoma	M/27	Yes	None	PR	30		150+
16	Renal cell carcinoma	M/46	Yes	None	NR			120
17	Melanoma	M/36	No	None	NE		CNS progression	30
18	Renal cell carcinoma	M/57	Yes	None	NR			134+
19	Melanoma	F/50	No	None	NE		CNS progression	16
20	Melanoma	M/43	Yes	None	NR			30
21	Embryonal cell carcinoma	M/22	Yes	VBL, *Cis*-Pt, Bleo, VP-16	NC	60		70+

*V = vincristine; C = cyclophosphamide; ADR = doxorubicin; P = prednisone; MTX = methotrexate; ARA-C = cytarabine; BUS = busulfan; 6-MP = 6-mercaptopurine; *Cis*-Pt = cisplatin; VBL = vinblastine; Bleo = bleomycin; VP-16 = etoposide; MOPP = mechlorethamine, vincristine, procarbazine, prednisone; ABVD = doxorubicin, bleomycin, vinblastine, dacarbazine; RT = radiation therapy; L-ASP = L-asparaginase; DNR = daunorubicin.

†NE = not evaluable; NC = no change; NR = no response.

Several investigators have reported their results with the use of cytoreductive therapy combined with autologous bone marrow transplantation. In a study conducted by Spitzer et al (16), the preparative regimen consisted of cyclophosphamide (6 g/m^2), carmustine (300 mg/m^2), and etoposide (600 mg/m^2). The median number of days of neutropenia (< 500 cells/mm^3) was 15, while the median number of days of thrombocytopenia was 36. All patients in this study had fever while neutropenic and 60% of the patients had documented infections. Furthermore, two patients had hemorrhagic cystitis related to cyclophosphamide administration, and three patients had interstitial lung disease. The overall CR plus PR was 60%, with the median duration of response being 11 weeks. At the time of the report, one patient with localized oat cell carcinoma of the lung was surviving for > 1 year.

Appelbaum et al (9,10) reported their experience of

transplantation in patients with lymphoma. Their preparative regimen consisted of carmustine (200 mg/m^2), cytarabine (800 mg/m^2), cyclophosphamide (6400 mg/m^2), and thioguanine (800 mg/m^2) followed by autologous transplantation 36 hours after the last dose of chemotherapy. Twelve patients underwent autologous transplantation, of whom nine were evaluable. The median duration of neutropenia ($\leq$ 500 cells/mm^3) and thrombocytopenia ($\leq$ 50,000 cells/mm^3) was 20 and 24 days, respectively. All patients became febrile while neutropenic, lasting a median of 7 days. All patients had gastrointestinal toxic effects, while hemorrhagic cystitis occurred in five patients and cardiac failure occurred in two of the transplant patients. Of the nine evaluable patients, all had either a CR or a PR. However, only three patients (all with Burkitt's lymphoma) had a long duration of response lasting 38+, 28+, and 18+ months.

Douer et al (17) reported the results of 14 patients undergoing cytoreductive therapy and autologous bone marrow transplantation. The preparative regimen in this group consisted of cyclophosphamide (100–240 mg/kg), vinblastine (0.5–1.0 mg/kg), and total-body irradiation (8.0 Gy). In addition, nine patients also received doxorubicin (120–180 mg/kg). Seven patients died < 30 days after transplantation. All patients had mucositis, which was severe in eight. The median duration of neutropenia (< 500 cells/mm^3) was 19 days, and the median duration of thrombocytopenia (< 50,000 cells/mm^3) was 24 days. Acute cardiorespiratory failure developed in the seven evaluable patients. Although the response rate (CR + PR) for the seven evaluable patients was 100%, five of these patients relapsed within 6 months. Two patients (one with testicular carcinoma and one with non-Hodgkin's lymphoma) had an unmaintained remission for > 2 years.

Table 3 compares the marrow and nonmarrow toxic effects previously reported by other authors with those in this series. Our patients have had fewer days of neutropenia and thrombocytopenia. Furthermore, our patients had none of the serious nonmarrow toxic effects previously reported. None of our patients had cardiac failure, hemorrhagic cystitis, or interstitial lung disease. Although high-dose carmustine is associated with pulmonary toxicity, this relationship is strongest for doses > 1200 mg/m^2. Also, some synergism for pulmonary toxicity with the addition of cyclophosphamide is suggested by the literature (18). It is possible that pulmonary toxicity might have occurred had the patients in question survived for longer periods. Some of our patients had gastrointestinal toxic effects (nausea, mucositis, and diarrhea), but such effects were mild in most cases. This lower order of toxicity is probably a function of less aggressive regimens.

Although our response rate is slightly lower than that in some previously reported series, our short duration of response is consistent with previous findings. This low response rate may be a function of the differences in patient population or of our preparative regimen. We, like others, have few patients with long unmaintained remissions.

It is possible that we have not reached the optimal dose levels for our various regimens. As has been previously suggested (1,2), the greater the dose, the greater the antitumor effect. However, this does not hold true in all cases (19). As has been previously shown, regimens more aggressive than the ones used in our study were associated with greater toxic effects while pro-

TABLE 3.—Comparison of marrow and nonmarrow toxicity

Toxic effect	Investigators (ref No.)			
	Spitzer et al (16)	Appelbaum et al (9,10)	Douer et al (17)	Roswell Park Memorial Institute
Median No. of days				
Neutropenia (< 500 cells/mm^3)	15	20	19	11
Thrombocytopenia (< 50,000 cells/mm^3)	36	24	24	14
Fever		7		10
No. of patients with—				
Hemorrhagic cystitis	2	5		0
Interstitial lung disease	3			0
Cardiac failure		2	7	0

ducing equivalent benefits. We therefore believe that one way of increasing the therapeutic index would be to deliver high-dose cytoreductive therapy early in the course of the disease so that the tumor burden is lower (and the cells are sensitive) when therapy is initiated. Also, when the tumor burden is low, there is greater likelihood that more cells will be in the log phase of proliferation (1); more cells will be in cycle and therefore more susceptible to cell cycle-specific cytotoxic agents.

We believe that high-dose cytoreductive therapy followed by reinfusion of cryopreserved autologous bone marrow can be easily performed with minimal toxicity; at most, the toxicity seen would be of the same order as that associated with first remission-induction therapy in acute myelocytic leukemia. Our responses in end-stage patients, while not of long duration or marked degree, encourage further study in these areas. Patients should be considered for autologous transplantation prior to extensive conventional therapy or after they have been rendered clinically "disease-free" but have an extremely high likelihood of disease recurrence.

REFERENCES

1. Skipper HE, Schabel FM, Jr, and Wilcot WS. Experimental evaluation of potential anticancer agents. XIII. On the criteria and kinetics associated with "curability" of experimental leukemia. Cancer Chemother Rep 35:3–111, 1964.
2. Frei E, III, and Canellos GP. Dose: a critical factor in cancer chemotherapy. Am J Med 69:586–594, 1980.
3. Bodey GP, Buckley M, Sathe YS, et al. Quantitative relationships between circulating leukocytes and infection in patients with leukemia. Ann Intern Med 64:328, 1966.
4. Cavins JA, Kasakura S, Thomas ED, et al. Recovery of lethally irradiated dogs following infusion of autologous marrow stored at low temperature in dimethylsulfoxide. Blood 20:730–734, 1962.
5. Cavins JA, Scheer SC, Thomas ED, et al. The recovery of lethally irradiated dogs given infusions of autologous leukocytes preserved at –80°C. Blood 23:38–43, 1964.
6. Lochte ML, Jr, Kasakura S, Koretzky M, et al. Infusion of marrow in the mouse and dog after thio-TEPA. Blood 21:424–428, 1963.
7. Epstein RB, Storb A, Clift RA, et al. Autologous bone marrow grafts in dogs treated with lethal doses of cyclophosphamide. Cancer Res 29:1072–1075, 1969.
8. Tobias JS, Weiner RS, Griffiths CT, et al. Cryopreserved autologous marrow infusion following high dose cancer chemotherapy. Eur J Cancer 13:269–277, 1977.
9. Appelbaum FR, Deisseroth AB, Graw RG, et al. Prolonged complete remission following high dose chemotherapy of Burkitt's lymphoma in relapse. Cancer 41:1059–1063, 1978.
10. Appelbaum FR, Herzig GP, Ziegler JG, et al. Successful engraftment of cryopreserved autologous bone marrow in patients with malignant lymphoma. Blood 52:85–95, 1978.
11. Buckner CD, Rudolph RH, Fefer A, et al. High dose cyclophosphamide therapy for malignant disease: toxicity, tumor response, and the effect of stored autologous marrow. Cancer 29:357–365, 1972.
12. Dicke KA, McCredie KB, Spitzer G, et al. Autologous bone marrow transplantation in patients with adult acute leukemia in relapse. Transplantation 26:169–173, 1978.
13. Graze PR, Wells JR, Ho W, et al. Successful engraftment of cryopreserved autologous bone marrow stem cells in man. Transplantation 27:142–145, 1979.
14. Dicke KA, Spitzer G, and Zender AR. Autologous bone marrow transplantation in relapsed adult acute leukemia and solid tumors. Transplant Proc 11:212–214, 1979.
15. Spitzer G, Dicke KA, and Verma DS. High-dose BCNU therapy with autologous bone marrow infusion: preliminary observations. Cancer Treat Rep 63:1257–1264, 1979.
16. Spitzer G, Dicke KA, and Litam J. High-dose combination chemotherapy with autologous bone marrow transplantation in adult solid tumors. Cancer 45:3075–3085, 1980.
17. Douer D, Champlin RE, and Ho W. High dose combined modality therapy and autologous bone marrow transplantation in resistant cancer. Am J Med 71:973–976, 1981.
18. Weiss RB, Poster DS, and Penta JS. The nitrosoureas and pulmonary toxicity. Cancer Treat Rev 8:111–125, 1981.
19. O'Bryan RM, Baker LM, and Gottlieb JE. Dose response evaluation of adriamycin in human neoplasia. Cancer 39:1940–1948, 1977.

Phase I Trial of 10-Deaza-aminopterin in Patients With Advanced Cancer[1,2]

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The clinical effects of 10-deaza-aminopterin, an inhibitor of dihydrofolate reductase with a better therapeutic index against several murine tumors than that of methotrexate, were examined during the course of a phase I study in patients with advanced malignant neoplasms. Three escalating dose schedules were explored: single iv injections once daily, single iv injections twice weekly, and continuous infusion. The maximum tolerated doses were: single injections at a dose of 7 mg/m²/day for 5 days; single injections at a dose of 15 mg/m² twice weekly for four to six doses; and continuous infusion at a dose of 3 mg/m²/day for 5–6 days in patients with solid tumors and until bone marrow hypoplasia in patients with leukemia. Mucositis was dose-limiting in all schedules. Occasionally, mild leukopenia, thrombocytopenia, and skin rash were noted. A minor antitumor response was seen in a patient with gallbladder carcinoma. Marked leukemic cell kill was observed in several patients with acute leukemia or blastic phase of chronic myelogenous leukemia. Disease-oriented phase II trials are planned at this Center for several tumor varieties. [Cancer Treat Rep 67:149–154, 1983]

On the basis of results obtained in murine tumor models, it has been proposed (1–5) that antifolate drugs of increased therapeutic efficacy can be obtained by detailed analysis of the structural characteristics that materially affect the following parameters: (*a*) drug transport into and out of neoplastic and nonneoplastic tissue, (*b*) drug binding to dihydrofolate reductase, and (*c*) rates of drug clearance from plasma. In a series of studies, Sirotnak and co-workers (1–5) established that structural modifications at the 10 position of aminopterin remarkably altered drug transport characteristics without greatly influencing the affinity of these agents for dihydrofolate reductase. Several of the 10-modified derivatives have significantly enhanced therapeutic activity against a series of transplanted murine tumors (6,7); 10-deaza-aminopterin (10-DAAM; fig 1) is the first of these drugs to be extended to clinical trial.

Detailed in vivo analysis of the factors responsible for the success of antifolate therapy in these murine tumor systems revealed that significantly prolonged retention of free antifolate (ie, in excess of the dihydrofolate reductase binding capacity) in the tumor cells beyond that occurring in the small intestine (the dose-limiting host tissue) was predictive of therapeutic utility (1,2). The factors responsible for this selective prolonged retention by the cancer cells were examined in vivo in terms of the influx K_m and V_{max} and the rates of efflux for various cell lines and for crypt cells of small intestines. The relative K_ms for tumor cells and gut cells were identified as the key kinetic parameters that were predictive of therapeutic utility for aminopterin, methotrexate (10-methylaminopterin), and 10-DAAM (6).

Our phase I development of 10-DAAM has been focused on low or "conventional" dosages rather than high dosage with leucovorin rescue, because 10-DAAM has shown improved therapeutic properties in mice when conventional dosages were used (6). Furthermore, the in vivo data suggest that very high plasma concentrations of the drug would forfeit the therapeutic advantage inherent in the favorable influx K_m relationships

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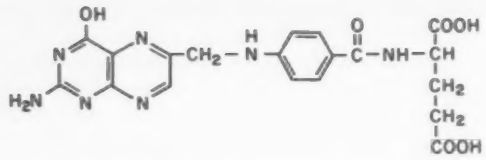

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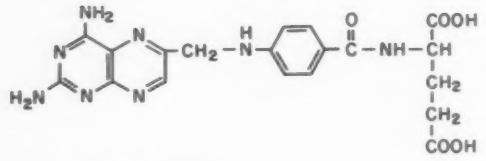

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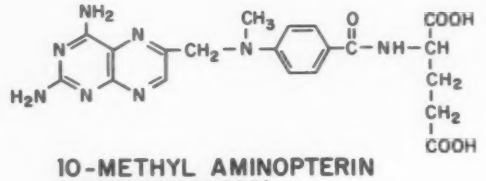
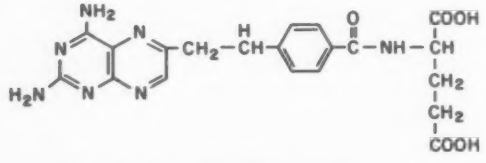


10-METHYL AMINOPTERIN
(METHOTREXATE)



10-DEAZA-AMINOPTERIN

FIGURE 1.—Structures of folic acid, aminopterin, metnotrexate, and 10-DAAM.

between gut and cancer cells. The starting dose for initial studies, 1.0 mg/m²/day × 5, was one-third the toxic dose low in the dog. In this communication we describe the phase I experience with three iv injection schedules: daily × 5, twice weekly, and continuous infusion.

MATERIALS AND METHODS

All patients entered in this study had histologically proven advanced cancer. They had become refractory to standard therapy or had neoplastic disease for which there is no effective therapy. No patient had received other therapy for at least 4 weeks prior to receiving 10-DAAM. All nonleukemic patients had acceptable hematologic status (wbc count ≥ 4000/mm³ and platelet count ≥ 120,000/mm³, serum creatinine ≤ 2.0, and with one exception, bilirubin ≤ 1.5 mg/dl). Informed consent for administration of this drug was obtained from all patients prior to entry in the study.

10-DAAM was supplied as a dry powder in the free acid form by Dr. Joseph I. DeGraw, Department of Pharmaceutical Chemistry, Stanford Research Institute, Menlo Park, CA. It was dissolved by suspension in sterile saline and brought into solution as the pH was adjusted to 7 with the addition of 0.1 N NaOH. The drug was sterilized by filtration through a 0.2-μm bacterial retention filter. The drug was administered to patients by single direct iv injection over 10 minutes or by continuous 24-hour infusion in 5% dextrose in water through a central venous catheter and an IVAC pump. The daily × 5 schedule was initiated first; the lowest dose level was one-third the toxic dose low in the most sensitive animal (the dog) in the preclinical studies.

Prior to the start of chemotherapy, all patients had a complete history, a physical examination, and a laboratory evaluation. The laboratory evaluation included automated blood cell count; differential count; 5′-nucleotidase; serum electrolytes; glucose; prothrombin time; biochemical screening profile (BUN, uric acid, calcium, total bilirubin, total protein, albumin, alkaline phosphatase, SGOT, LDH, and phosphorus); creatinine clearance; urinalysis; electrocardiogram; and chest X-ray. All patients were monitored for evidence of hematopoietic, gastrointestinal, hepatic, renal, cutaneous, and central nervous system toxic effects. Weekly follow-up studies included automated blood cell count, screening profile, 5′-nucleotidase, and serum creatinine. These studies were also repeated at the end of each course. The chest X-ray was repeated monthly.

Patients with leukemia were monitored by daily blood cell counts and by an assessment of bone marrow cellularity; bone marrow was obtained by aspiration or biopsy every 7–10 days. Drug administration was continued in these patients until bone marrow hypoplasia was seen or until moderate toxicity supervened. Patients with leukemia were considered evaluable for adequacy of the trial if mucositis occurred or if the peripheral blast count increased > 30% from the initial value despite treatment.

Nonhematologic toxicity was graded according to the following convention: 1+ = present but not disabling; 2+ = disabling but not requiring supportive medical treatment; 3+ = both disabling and requiring supportive medical management; and 4+ = life-threatening, requiring hospitalization.

Patients showing no evidence of toxic effects 3 weeks after the completion of a course of 10-DAAM were eligible to receive a second course at an escalated dose level.

Response criteria were defined as follows: *complete remission (CR)*—the disappearance of all clinical evidence of active tumor for a minimum of 4 weeks and freedom from all symptoms; *partial remission (PR)*—a 50% decrease in the sum of the products of the greatest perpendicular diameters of all measurable lesions, without evidence of increase in the size of any lesion or the appearance of new lesions; *minor response*—an objective ($\geq$ 25%) decrease in measurable disease, but less than required for PR; *no change*—a $<$ 25% decrease in tumor size; and *disease progression*—an objective increase ($>$ 25%) in measurable disease. Responses in patients with leukemia were assessed as previously described (8).

RESULTS

Sixty-two adult patients were entered in this study; 55 were evaluable (table 1). When 10-DAAM was given by single injection, hematologic toxicity was very mild; leukopenia occurred less frequently than thrombocytopenia (table 2). Two patients required platelet transfusions and leucovorin rescue for minor but persistent bleeding. Hematologic depression was more frequent in the continuous-infusion regimen in nonleukemic patients.

Stomatitis proved to be the dose-limiting toxic effect in both single iv injection regimens; ie, the daily × 5 days and twice weekly schedules (table 3). Stomatitis and/or hematologic toxicity were dose-limiting in the 24-hour continuous-infusion regimen, in both leukemic and nonleukemic patients (tables 4 and 5).

Other acute side effects of 10-DAAM were generally mild and infrequent. Cutaneous toxicity consisting of a maculoerythematous rash was noted in four patients receiving the daily × 5 regimen. Nausea and vomiting occurred only rarely. Occasionally, patients had a transient rise in SGOT without elevation of other liver function measurements. BUN and creatinine were not noted to rise during the course of therapy or with subsequent courses of therapy.

Antineoplastic effects.—Although no consistent therapeutic effect was seen in evaluable patients with solid tumors who experienced at least 1+ toxic effects in this trial, one patient with gallbladder cancer who was treated with the daily × 5 regimen had clearing of jaundice and marked improvement in all liver function tests after receiving 10-DAAM on two occasions. One patient with adenocarcinoma of the lung treated on the same schedule had at least a 25% decrease in the size of a meas-

TABLE 1.—Characteristics of 55 evaluable adult patients during 59 courses*

Characteristic	Schedule			
	Daily × 5 iv	Twice weekly iv	Continuous infusion	
			Leukemia	Nonleukemia
Diagnosis				
Leukemia			15	
Breast cancer	1	3		3
Lymphocytic lymphoma				2
Renal cancer				2
Mycosis fungoides				1
Hodgkin's disease				1
Lung cancer	8			2
Head and neck cancer		3		1
Sarcoma	2	2		
Melanoma	2	1		
Colon cancer	5	1		
Gastric cancer	1			
Adenocarcinoma, unknown primary	1			
Gallbladder cancer	1			
Bladder cancer				1
Karnofsky performance status (%)				
Median	70	70	60	70
Range	40–90	40–90	30–90	40–90
No. of patients with prior methotrexate treatment	4	3	10	10
Creatinine clearance (ml/min)				
Median	59.0	65	62.5	52.15
Range	10–87.1	40–82.0	45–96	41.5–59.0

*Unless specified otherwise, values = No. of courses.

TABLE 2.—Myelosuppressive effects of 10-DAAM in adult patients, by regimen

Dose (mg/m^2)	No. of patients entered	No. of evaluable patients	Wbc count ($\times 10^3$ cells/mm^3) Nadir range	Wbc count ($\times 10^3$ cells/mm^3) Median nadir (Day)	Platelet count ($\times 10^3$ cells/mm^3) Nadir range	Platelet count ($\times 10^3$ cells/mm^3) Median nadir (Day)
			Daily × 5 days			
1	4	3	5.9–8.7	6.7 (14)	294–450	356.0 (14)
2	3	3	3.8–9.6	4.5 (4)	33.1–185	107.0 (10)
3	3	3	4.2–6.1	5.15 (7)	90–270	161 (13.5)
4	3	3	3.2–7.7	4.4 (8)	124–387	290 (7)
5	2	2	3.5–14.1	6.4 (17)	90–155	139 (11)
6	6	6	3.0–8.9	5.4 (10)	16–325	183 (10)
7	6	5	4–6.3	5.15 (11.5)	10–289	154.5 (12)
			Twice weekly			
15	6	6	4.4–8.7	5.6 (6)	129–498	256 (13)
18	4	3	3.8–5.5	5.3 (15)	72–177	80 (11)
			24-hr infusion (nonleukemic patients)			
0.75	5	5	2.6–10.9	4.1 (8)	98–247	124 (9)
1	5	3	4.6–9.2	5.3 (2)	117–174	135 (8)
1.5	6	5	0.1–5.1	3.1 (16)	4.0–309	201 (15)
2	1	1	3.2	3.2 (8)	56	56 (8)
3	1	1	0.5	0.5 (8)	16	16 (11)

TABLE 3.—Toxicity associated with 10-DAAM given by single injections (daily × 5 and twice weekly) in adult patients

Dose (mg/m^2)	No. of evaluable patients	Mucositis 0	1+	2+	3+	4+	Cutaneous toxicity 0	1+	2+	3+	4+
					Daily × 5						
1	3	3					3				
2	3	2	1				3				
3	3	1	1	1			2	1			
4	3		1				2	1			
5	2	2	3				2				
6	6	3		1	2		6				
7	5	1			2	2	3		2		
					Twice weekly						
15	6		1	4	1		6				
18	3	1	1		1		3				

TABLE 4.—Toxicity associated with 10-DAAM given by 24-hr continuous infusion in nonleukemic adult patients

Dose (mg/m^2/day)	No. of evaluable patients	Mucositis 0	1+	2+	3+	4+	Cutaneous toxicity 0	1+	2+	3+	4+
0.6	1	1					1				
0.75	5	5					5				
1.0	3	3					3				
1.5	5		1	1	2	1	5				
2.0	1					1	1				
3.0	1					1	1				

TABLE 5.—Toxic and therapeutic effects of 10-DAAM administered by continuous iv infusion in adult patients with leukemia

Dose (mg/m²/day)	Diagnosis*	Duration of infusion (days)	Effect on wbc count†	Muscositis grade
0.75	AML/ALL	8	↑	2+
	AMMoL	10	↑	0
1.5	ALL	5	↑	0
	ALL	5	↓	3+
	CML-Bl	7	↓	1+
	AML	7	↓	4+
	ALL	7	↑	0
	CML-Bl	9	↓	2+
	CML-Bl	9	↓	0
	AML	13	↓	3+
3.0	AML	3	0	0
	AML	5	↓	0
	AML/ALL	6	0	3+
	AML	6	↑	3+
	AML	7	↓	3+
6.0	AML	4	0	4+
	AML	4	0	4+

*AML = acute myeloblastic leukemia; ALL = acute lymphoblastic leukemia; AML/ALL = biphenotypic leukemia; AMMoL = acute myelomonoblastic leukemia; CML-Bl = chronic myelocytic leukemia, blast phase.
† ↓ indicates at least a log-fold reduction in total peripheral wbc counts; ↑, a ≥ 30% increase in total wbcs above baseline. Intermediate changes are recorded as 0.

urable subcutaneous nodule. A patient with lymphocytic lymphoma treated on the 24-hour continuous-infusion schedule had a 25% regression in the size of his measurable cervical lymphadenopathy.

Seventeen courses of therapy were administered to 15 adult patients with acute leukemia or with CML-Bl. The therapeutic and toxic effects of 10-DAAM administered by continuous infusion in this patient population are presented in table 5. Major leukemic cell kill (defined as > 1 log reduction in the peripheral wbc count) occurred in nine of 12 evaluable patients who received 10-DAAM as a continuous infusion at either 1.5 or 3.0 mg/m²/day. Bone marrow hypoplasia occurred in two patients, one with AML and one with CML-Bl. Although neither patient achieved remission, treatment with 10-DAAM produced symptomatic improvement with a concomitant increase in performance status which lasted 4–6 weeks.

DISCUSSION

The real but limited utility of methotrexate in the treatment of cancer in man has stimulated research directed toward development of more effective antifolate drugs. Development of resistance and initial nonresponsiveness have been linked to low drug accumulation within tumor tissues. 10-DAAM is one of a series of compounds with which it is hoped to achieve therapeutic advantage by selecting membrane transport characteristics, with regard to influx K_m, that permit ready entry of the drug into a number of types of neoplastic cell but that considerably reduce drug entry into gut cells. In transplanted murine tumor test systems, drug-induced injury of the small intestine constitutes the dose-limiting host toxicity for methotrexate analogs.

To provide flexibility for future trials, we have defined dose-effect relationships for 10-DAAM given according to three iv schedules: daily × 5, two times weekly, and continuous infusion. The data from this study indicate that the following are suitable dosages for phase II evaluation in the three schedules examined: iv injection daily × 5 at 7 mg/m²/day; iv injection twice weekly × 3 weeks at 15 mg/m²; and iv infusion for 5–7 days at 1.5 mg/m²/day.

We have observed similar, albeit not identical, toxic manifestations with all three dose schedules. The most sensitive nonneoplastic tissue was the mucous membrane of the oral pharynx. At dosages that produce moderate to severe stomatitis, only mild intestinal toxicity, manifested by diffuse abdominal pain and occasional diarrhea, was observed. Dermatitis, particularly involving the intertriginous areas, was seen only occasionally by patients on the twice weekly or daily × 5 schedule; it was observed in 40% of the patients given the maximum tolerated dose by continuous iv infusion but was not in itself dose-limiting.

The clinical toxic effects observed with both 10-DAAM and methotrexate are similar although the available data suggest that methotrexate produces more enterocolitis, with diarrhea and tenesmus, and more myelosuppres-

sion at toxic dosages than we have observed with 10-DAAM. Although it would probably require a randomized blind trial to establish this point securely, the available data are at least consistent with the idea that the intestinal sparing observed with the use of 10-DAAM in mice may occur also in man.

We chose the low-dose continuous infusion of 10-DAAM for the treatment of patients with leukemia in an effort to exploit the differences between the influx K_m shown for normal cells and tumor cells. It was hoped that this method would maximize the therapeutic index for this antifol relative to methotrexate, a drug with minimal activity as a single agent in leukemia. Although marked reductions in leukemic blasts were noted, the therapeutic index appeared quite narrow. Prolonged infusions (> 7 days) caused appreciable toxic effects and did not produce clinical remission of disease. It is conceivable that several short courses of 10-DAAM might be better tolerated and could be of greater clinical utility.

We expect to define more precisely the magnitude of antileukemic activity of 10-DAAM during a phase II trial of the drug, when it is administered earlier in the course of disease to patients in better condition.

REFERENCES

1. Chello PL, Sirotnak FM, Dorick DM, et al. Therapeutic relevance of differences in the structural specificity of the transport systems for folate analogs in L1210 tumor cells and in isolated murine intestinal epithelial cells. Cancer Res 37:4297–4303, 1977.
2. Sirotnak FM. Correlates of folate analog transport, pharmacokinetics and selective antitumor action. Pharmacol Ther 8:71–103, 1980.
3. Sirotnak FM, Chello PL, and Brockman RW. Potential for exploitation of transport systems in anticancer drug design. *In* Methods in Cancer Research (Busch H, and DeVita VT, eds). 1979, vol 16, pp 381–447.
4. Sirotnak FM, Chello PL, Piper JR, et al. Growth inhibitory transport and biochemical properties of the glutamyl and aspartyl peptides of methotrexate in L1210 leukemia cells in vitro. Biochem Pharmacol 27:1821–1925, 1978.
5. Sirotnak FM, DeGraw JI, and Chello PL. New folate analog development by an analysis of membrane transport specificity and tissue pharmacokinetics. *In* Current Chemotherapy. Washington, DC, American Society for Microbiology, 1978, pp 1128–1130.
6. Sirotnak FM, DeGraw JI, Chello PL, et al. Biochemical and pharmacologic properties of a new folate analog, 10-deaza-aminopterin, in mice. Cancer Treat Rep 66:351–358, 1982.
7. Sirotnak FM, DeGraw JI, Moccio DM, et al. Antitumor properties of a new folate analog, 10-deaza-aminopterin, in mice. Cancer Treat Rep 62:1047–1052, 1978.
8. Arlin ZA, Sklaroff RB, Gee TS, et al. Phase I and II trial of 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide in patients with acute leukemia. Cancer Res 40:3304–3306, 1980.

Phase II Trial of the Dopaminergic Inhibitor Pimozide in Previously Treated Melanoma Patients[1,2,3]

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Pimozide, a potent neuroleptic which inhibits the release of pituitary releasing factors and is an effective dopamine antagonist, was administered to 30 patients with previously treated metastatic melanoma. Six patients were inevaluable because of poor drug tolerance (two), disease progression within 1 week and death within 2 weeks (three), and death from other causes (one). Among the 24 evaluable patients, two had complete response, two had partial response, and two had disease stabilization. Responses were observed in soft tissue, lymph nodes, liver, and lung. Toxic effects consisted of extrapyramidal manifestations in nine patients and malaise in seven. Pimozide has activity in patients with previously treated metastatic melanoma (17% response rate in evaluable patients) and merits consideration of further study in combination regimens. [Cancer Treat Rep 67:155–157, 1983]

The management of metastatic melanoma has been difficult with standard chemotherapeutic agents; the most effective drugs, dacarbazine and semustine, have objective response rates of 15%–20% and short response durations (1). Randomized trials have suggested no benefit of combination chemotherapy compared to single agents.

Many melanomas contain the unique biochemical pathway terminating in the synthesis of melanin. In a series of reports, Wick and co-workers (2–4) have demonstrated that precursors in the melanin synthesis pathway can inhibit the growth of some murine melanomas. Wick (5) has subsequently demonstrated that a high-dose infusion of dopamine, the first step in the branch pathway leading to catecholamine synthesis, can significantly reduce the labeling index of human melanoma cells in vivo, thus suggesting a biochemical inhibition of human melanoma. Varga et al (6) have shown that melanocyte-stimulating hormone (MSH) can modulate murine melanoma cell growth. Although MSH is not the primary physiologic regulator of melanin synthesis in man, at least some human melanomas appear to be regulated by MSH in vitro (7–9). In man, MSH activity appears to reside in the ACTH and β-lipotropin molecules (10). Recent investigations (11) suggest that pimozide, a drug that blocks melanin synthesis and inhibits the release of these pituitary releasing factors, can inhibit macromolecular synthesis in cultured murine melanoma cells and prolong the lives of mice bearing transplants of those melanomas.

The purpose of this study was to investigate the activity and toxicity of pimozide in previously treated patients with metastatic melanoma.

PATIENT SELECTION AND METHODS

Patients with metastatic melanoma and measurable or evaluable (in the case of bone) disease were eligible for treatment with pimozide. Most patients had been previously treated for metastatic disease with a variety

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of drugs, alone or in combination; only two patients were entered in the trial without previous chemotherapy.

Before entry in the protocol, patients underwent a complete physical examination and had the following laboratory values determined: hemoglobin, hematocrit, wbc count, platelet count, LDH, SGOT, alkaline phosphatase, bilirubin, BUN, creatinine, and calcium. Chest radiography and, when appropriate, liver, brain, and bone radioisotope scans and computed tomography were performed. Measurements of all evaluable lesions were recorded prior to the beginning of pimozide treatment. Previous chemotherapy had to have been discontinued for at least 3 weeks (at least 6 weeks for nitrosourea).

Criteria for ineligibility included: no prior chemotherapy (until the last two patients were entered), nonmeasurable or inevaluable disease, severe depression, extrapyramidal disorders, or concurrent administration of major tranquilizer drugs. Informed consent was obtained from all patients.

Pimozide (supplied by McNeil Laboratories, Spring House, PA) was administered orally at a dosage of 8 mg/m^2/day, to a maximum of 12 mg/day, as a single dose at bedtime. This dosage was based on studies in a patient with generalized lipodystrophy, demonstrating inhibition of hypothalamic releasing factors and depigmentation at that dose (12). Patients received the drug for at least 1 month or until disease progression. They were followed for new areas of metastases and had monthly evaluations of tumor sites.

Complete response (CR) was defined as the complete disappearance of all clinically detectable malignant disease without development of new malignant lesions, lasting at least 4 weeks. Patients with bony metastases were required to have normalization of all bone X-rays or bone scans. Partial response (PR) was defined as a $\geq$ 50% decrease in tumor size in $\geq$ 50% of the involved organ sites, lasting for at least 4 weeks without increase in size of any area of known malignant disease or appearance of new areas of malignant disease. Disease stabilization was defined as no significant change in measurable disease for at least 8 weeks (12 weeks for bony metastases). Progressive disease was defined as a significant ($\geq$ 25%) increase in size of lesions present at the initiation of therapy or appearance of new lesions not previously known to be present.

RESULTS

Pimozide was administered to 30 patients at four institutions. Patient characteristics are summarized in table 1. Six patients were inevaluable: three had rapid disease progression within 1 week and died within another week; one died 1 week after beginning pimozide, of severe hypoglycemia due to acute alcoholism; and two did not tolerate the drug because of severe malaise and restlessness and ceased taking pimozide within 1 week. Of the 24 evaluable patients, four (17%) had responses (2 CR and 2 PR; 95% confidence limits, 5%–37%) and two had disease stabilization.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	30
Male/female	16/14
Median age in yrs (range): 51 (21–82)	
Metastatic sites	
Skin and subcutaneous	20
Lymph nodes	12
Lung	11
Liver	9
Brain	3
Bone	3
Other	5
No. of organ sites	
1	6
2	14
3	8
> 3	2
Performance status*	
0	10
1	17
2	2
3	0
4	1
No. of previous chemotherapeutic regimens†	
0	2
1	19
2	6
3	0
4	3

*Eastern Cooperative Oncology Group criteria.
†1–4 drugs/regimen.

CR was observed in one patient with a pulmonary metastasis (13) and in one who had a primary anal melanoma with biopsy proven nodal and liver metastases. PR was observed in one patient with multiple pulmonary and hepatic metastases and in one with soft tissue and hepatic metastases. Response durations were 36+, 8, 14, and 11+ months, respectively. The two patients with disease stabilization had disease progression after 4 and 5 months.

Toxic effects were tolerable in most patients (table 2). Among the 24 evaluable patients, seven complained of malaise and nine demonstrated some degree of extrapyramidal manifestations resembling Parkinson's disease. When the drug dose was decreased in six patients, their symptoms were decreased and three patients could tolerate subsequent increased doses of pimozide without recurrence of malaise or choreoathetoid movements. Four patients reported insomnia, and four had both malaise and xerostomia. Weakness could

TABLE 2.—Toxicity

Toxic effect	No. of patients
Extrapyramidal	9
Malaise	7
Insomnia	4
Xerostomia	4
"Weakness"	2

not be definitely attributed to pimozide or to progressive disease. No patient had myelosuppression, nausea, vomiting, or skin rash.

DISCUSSION

Melanoma represents a malignant degeneration of melanocytes, cells derived from the neural crest. Many melanomas retain the biochemical pathway which converts phenylalanine to tyrosine to dopa, with formation of dopaquinone and subsequent spontaneous polymerization to form melanin. Other investigators (2–4) have shown that high concentrations of dopa, dopa methylester, or dopamine can inhibit macromolecular precursor incorporation in some murine melanomas in vitro and prolong life in vivo. Our previous work has demonstrated that the antidopamine drug pimozide will inhibit macromolecular precursor incorporation in vitro in a dose-response fashion in melanin-producing murine melanomas and prolong life in vivo, even in tumors on which dopa, dopa methylester, and dopamine had no effect (11). These results were found to correlate with the presence of specific membrane-bound receptors for dopamine; thus, the effects of pimozide may be analogous to those of antiestrogens in patients with advanced breast cancer whose tumors are estrogen receptor-positive. Furthermore, MSH has been shown to regulate some murine (6) and human (7–9) melanoma cell growth. The demonstration that pimozide inhibited the release of pituitary releasing factors in man, with one effect being depigmentation via inhibition of polypeptides having MSH activity (12), provided further scientific rationale to study pimozide in patients with metastatic melanoma.

Twenty-four previously treated patients received pimozide for at least 4 weeks and could be evaluated for antitumor efficacy of the drug. There were four (17%) responses (2 CR, 2 PR) of visceral as well as soft tissue disease. This compares favorably to other single drugs and drug combinations in which the expected response rate of previously treated patients is only about 5%. In general, the toxicity was manageable, although two patients could not tolerate the drug because of severe malaise. Side effects among the other 24 patients included malaise in seven and extrapyramidal manifestations in nine; decreasing the dose of pimozide resulted in amelioration of symptoms in most patients. No bone marrow, pulmonary, hepatic, or gastrointestinal toxicity has been observed at the dosages utilized in this study. Thus, the drug was fairly well tolerated by most patients.

This phase II trial of pimozide in patients with previously treated melanoma suggests that the drug has activity in this disease. Because the major toxic effects (ie, malaise and extrapyramidal effects) do not overlap with those of standard chemotherapeutic agents and the mechanism of action is also different, pimozide may prove to be a useful drug in combination regimens.

REFERENCES

1. COSTANZA ME, NATHANSON L, SCHOENFELD D, ET AL. Results with methyl-CCNU and DTIC in metastatic melanoma. Cancer 40:1010–1015, 1977.
2. WICK MM, BYERS L, and FREI E. L-Dopa: selective toxicity for melanoma cells in vitro. Science 197:468–469, 1977.
3. WICK MM. L-Dopa methylester as a new antitumor agent. Nature 269:512–513, 1977.
4. ———. Dopamine: a novel antitumor agent active against B-16 melanoma in vivo. J Invest Dermatol 71:163–164, 1978.
5. ———. Therapeutic effect of dopamine infusion on human malignant melanoma. Cancer Treat Rep 66:1657–1659, 1982.
6. VARGA JM, DIPASQUALE A, PAWELEK J, ET AL. Regulation of melanocyte stimulating hormone action at the receptor level: discontinuous binding of hormone to synchronized mouse melanoma cells during the cell cycle. Proc Natl Acad Sci USA 71:1590–1593, 1974.
7. LEGROS F, COEL J, DOYEN A, ET AL. α-Melanocyte-stimulating hormone binding and biological activity in a human melanoma cell line. Cancer Res 41:1539–1544, 1981.
8. FULLER BB, and MEYSKENS FL. Endocrine responsiveness in human melanocytes and melanoma cells in culture. JNCI 66:799–802, 1981.
9. MEYSKENS FL. The endocrinology of malignant melanoma. Rev Endocrine-Related Cancer 9:5–13, 1981.
10. LOWRY PJ, and SCOTT AP. Structural relationships and biosynthesis of corticotropin, lipotropin, and melanotropin. Front Hormone Res 4:11–17, 1977.
11. KRUMMEL TM, NEIFELD JP, and TAUB RN. Effects of dopamine agonists and antagonists on murine melanoma: correlation with dopamine binding activity. Cancer 49:1178–1184, 1982.
12. CORBIN A, UPTON GV, MABRY CC, ET AL. Diencephalic involvement in generalized lipodystrophy: rationale and treatment with the neuroleptic agent, Pimozide. Acta Endocrinol 77:209–220, 1974.
13. TAUB RN, and BAKER MA. Treatment of metastatic malignant melanoma with pimozide. Lancet 1:605, 1979.

Phase I Study of Tricyclic Nucleoside Phosphate[1,2]

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A phase I study of tricyclic nucleoside phosphate was conducted in 20 adults with advanced cancer. Tricyclic nucleoside phosphate was given as an iv infusion over 15 minutes once every 3 weeks; the doses ranged from 25 to 350 mg/m^2. Beginning at a dose of 250 mg/m^2, hyperglycemia and elevation of hepatocellular enzymes were observed; at a dose of 350 mg/m^2, two patients developed irreversible liver damage. Patients at all dose levels experienced reduction in serum phosphorus; reduction of serum calcium was noted only with the two highest doses. Nausea and vomiting occurred occasionally. Myelosuppression was not a prominent toxic effect. No major therapeutic responses were noted. Further clinical trials employing this schedule are probably not warranted. [Cancer Treat Rep 67:159–162, 1983]

In 1971, Schram and Townsend (1) reported the synthesis of a new tricyclic nucleoside (TCN, NSC-154020) which is structurally similar to several biologically active 7-deazapurine nucleosides. TCN rapidly enters cells (2) via a nucleoside transport system (3) and is phosphorylated by adenosine kinase (4,5) to TCN-5′-monophosphate (TCN-P, NSC-280594; fig 1). Unlike other nucleosides currently in clinical use, TCN-P is not phosphorylated beyond the monophosphate level (5,6) and is not incorporated into polynucleotides (5). The mechanism of action may be inhibition of DNA synthesis with selective toxicity to S-phase cells (7).

TCN is cytotoxic in vitro to L1210 leukemia and Novikoff hepatoma cells, and it possesses antitumor activity against murine L1210 leukemia (8). TCN and TCN-P are equally cytotoxic in vitro and both are effective against murine L1210 leukemia (8). In fact, TCN-P is probably dephosphorylated to TCN by a cellular ecto-5′-nucleotidase prior to its entry into cells, and then rephosphorylated by adenosine kinase to TCN-P, the active form of the drug (9). In addition to its activity against L1210, TCN-P is active against the MX-1 human mammary carcinoma, the $CD8F_1$ murine mammary tumor, and P388 leukemia (10). Because it is water-soluble, TCN-P is suitable for iv administration.

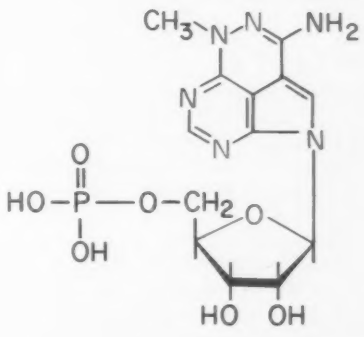

FIGURE 1.—Structure of TCN-P.

Initial animal toxicology studies were performed in mice, with confirmatory studies subsequently done in dogs. The toxic effects in both species included bloody diarrhea, prostration, and myelosuppression, as well as hepatic, adrenal, renal, and testicular damage.

This phase I study was conducted to identify a dose of TCN-P suitable for iv administration to patients every 3–4 weeks.

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PATIENTS AND METHODS

Each patient treated has a histologically confirmed diagnosis of malignancy. Criteria for eligibility included a performance status of at least 50% (Karnofsky scale), a wbc count > 4000/mm^3, a platelet count > 150,000/mm^3, normal serum bilirubin (< 1.5 mg/dl), and normal serum creatinine (< 1.5 mg/dl). Informed consent was obtained from each patient prior to the initiation of treatment. All patients had previously been treated with conventional therapeutic modalities. No patient had received radiotherapy or chemotherapy within 3 weeks prior to treatment with TCN-P. The characteristics of the 20 patients entered in this study are presented in table 1.

TABLE 1.—TCN-P: characteristics of 20 patients

Characteristic	No. of patients
Age in yrs	
Range: 37–72	
Median: 58	
Performance status (Karnofsky)	
Range: 50–100	
Median: 70	
Sex	
Male	11
Female	9
Prior therapy	
Chemotherapy	6
Radiation therapy	1
Chemotherapy and radiation therapy	13
Pathologic diagnosis	
Breast (adenocarcinoma)	1
Head/neck (epidermoid carcinoma)	4
Lung	6
Adenocarcinoma	4
Epidermoid carcinoma	2
Pancreas (adenocarcinoma)	1
Thyroid (medullary carcinoma)	2
Primary undetermined (adenocarcinoma)	4
Melanoma	2

Each patient had an initial complete history and physical examination. The following baseline blood tests were performed on all patients: cbc, platelet count, prothrombin time, serum electrolytes, creatinine, and a biochemical profile which included BUN, glucose, SGOT, LDH, total bilirubin, alkaline phosphatase, calcium, phosphorus, total protein, and albumin. In addition, for patients treated at the highest dose level, baseline insulin levels and 2-hour postprandial blood glucose levels were measured. A chest roentgenogram was required for each patient; radionuclide scans were performed only when clinically indicated. An attempt was made to identify evaluable parameters of disease in all patients, but the absence of measurable lesions was not used to exclude patients from this phase I study.

TCN-P was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD), in 10-ml vials containing 50 mg of anhydrous base, mannitol (100 mg), and sodium hydroxide to adjust the pH to 6.0–7.5. After reconstitution with sterile water for injection USP, TCN-P was administered by iv infusion over 15 minutes. Patients were observed for at least 3 weeks before another dose of the drug was administered.

The initial dose was 25 mg/m^2, approximately 1/30 of the mouse LD10. At least three patients were treated at each dose level and were observed for 3 weeks before additional patients were treated with the next higher dose. At doses which produced definite toxic effects, at least five patients were treated. Patients who experienced no toxic effects after their first dose of the drug were eligible to receive a second dose of the drug at a higher dose level.

Initially, only hospitalized patients were treated; however, after it became clear that there were no acute, unanticipated side effects, outpatient treatment was started. A cbc, a platelet count, and a biochemical profile were obtained at least once weekly. Qualitative toxic effects were graded on a scale of 0–4+ defined as follows: 1+ = mild but definite toxicity that was not disabling; 2+ = moderate side effects that required no special supportive management; 3+ = moderate to severe toxicity that required supportive care; and 4+ = severe, potentially life-threatening reaction requiring hospitalization for management. Treatment was continued once every 3 weeks in the absence of dose-limiting toxicity or progressive disease.

RESULTS

Twenty patients were entered in this study. Two patients were considered inevaluable because of failure to undergo follow-up blood tests, although neither reported symptoms that were obviously related to TCN-P. Eighteen evaluable patients received a total of 27 doses of TCN-P.

The hematologic effects of treatment are outlined in table 2. Myelosuppression was uncommon and not clearly drug-related. A platelet count < 25,000/mm^3 was observed in four patients. Bone marrow aspirates from these patients showed normal numbers of megakaryocytes, erythroid hyperplasia, and increased numbers of histiocytes. Prothrombin and partial thromboplastin times were prolonged in all four patients, and were compatible with hepatic dysfunction or disseminated intravascular coagulation. All of these patients died shortly thereafter with progressive cancer.

The nonhematologic toxic effects are presented in table 3. After treating six patients with TCN-P at a dose of 250 mg/m^2 it appeared that the most prominent toxic effects were nausea, vomiting, and hyperglycemia. The

TABLE 2.—Phase I study of TCN-P: hematologic toxicity

Dose (mg/m²)	No. of patients— Entered	Evaluable	Wbc count nadir (× 10³/mm³) Range	Median	Platelet count nadir (× 10³/mm³) Range	Median
25	4	3	10.2–16.3	10.6	301–475	351
50	4	4	5.1–14.1	8.0	342–384	357
100	3	3	4.5–29.7	8.4	44–300	279
165	4	3	6.0–9.8	6.4	78–323	167
250	6	6	4.5–18.8	7.6	33–377	120
350	5	5	1.0–5.8	4.8	14–226	24

TABLE 3.—Phase I study of TCN-P: nonhematologic toxicity

Dose (mg/m²)	No. of evaluable patients	Maximum blood glucose (mg/dl) Range	Median	Lowest serum phosphorus (mg/dl) Range	Median	Lowest serum calcium (mg/dl) Range	Median	Maximum SGOT (units/L) Range	Median
25	3	89–257	114	2.3–3.0	2.8	8.3–11.4	9.6	12–51	13
50	4	81–169	134	1.1–3.5	2.7	9.5–10.6	9.7	11–440	44
100	3	97–193	106	2.3–3.1	3.0	8.7–10.0	9.1	20–71	59
165	3	103–184	130	2.7–3.3	2.9	7.5–9.6	9.3	23–55	40
250	6	112–1335	210	1.4–3.1	1.9	6.8–9.0	7.6	42–154	90
350	5	188–432	226	1.0–2.7	2.1	5.7–8.9	6.6	99–1565	220
Normal values		70–110		2.5–4.0		9.2–10.8		< 25	

hyperglycemia was transient and mild in five of the patients, beginning on the day after therapy and reaching a maximum at 7–10 days following treatment. One of these patients presented with severe hyperglycemic, hyperosmolar ketoacidosis 5 weeks after receiving TCN-P. His metabolic derangements were controlled with iv fluids, electrolytes, and insulin. This patient, who had no previous history of glucose intolerance, required insulin for an additional 12 weeks.

Patients treated with 250 mg/m² of TCN-P also experienced elevation of SGOT and LDH without hyperbilirubinemia. This was noted as early as 3 days after drug administration. Maximum elevation of SGOT generally occurred by Day 7 and started to return toward normal by Day 14. However, complete resolution of these abnormalities was not observed in all patients. Transient reduction of the serum phosphorus level was observed in all patients treated at this dose, and in three patients the serum phosphorus fell below the normal range. Changes in serum calcium levels, however, were not observed.

Anticipating that hyperglycemia might be dose-limiting, glucose and insulin levels were monitored closely for all patients treated at the next higher dose, 350 mg/m². Mild to moderate elevation of serum glucose was observed in all patients. Serum insulin levels were inappropriately low for the degree of hyperglycemia in these patients; however, only one patient at this dose level required exogenous insulin. Four of five patients treated at this dose level also experienced hypophosphatemia, and two of these developed hypocalcemia as well. All patients developed abnormal elevations of hepatic enzymes, and two of these patients had irreversible liver damage. One of the two patients was a 62-year-old woman with progressive medullary carcinoma of the thyroid who presented with hypocalcemia and tetany 3 weeks after receiving TCN-P. Even with aggressive treatment with calcium gluconate her hypocalcemia was never totally controlled, although urinary excretion of calcium remained minimal. Correction of her low serum magnesium level likewise did not affect the hypocalcemia. This patient developed progressive elevation of her SGOT, LDH, alkaline phosphatase, and serum bilirubin (maximum concentration, 16.8 mg/dl) and died with progressive disease and liver dysfunction. Permission for a full postmortem examination was not granted; however, consent was obtained for removal of a small piece of liver. The liver showed fatty changes and cholestasis; metastatic tumor was also identified in this limited material. The other patient was a 48-year-old man with advanced epidermoid carcinoma of the lung. He developed a transient increase in his liver enzymes and bilirubin after TCN-P, which began to return toward normal 7 days after he received the drug. During the following week, however, he was found to have a serum bilirubin of 5.2 mg/dl. This patient experienced progressive rise in hepatic enzymes and bilirubin until he died 8 weeks after receiving TCN-P. An autopsy revealed extensive tumor in the chest but no liver metastases. Fatty change and diffuse cholestasis were again

noted in the liver. There was no evidence of extrahepatic biliary obstruction. Drug-induced renal, adrenal, and pancreatic necrosis was not present, but significant autolysis had taken place. Both of these patients had prolonged thrombocytopenia and coagulation abnormalities compatible with liver damage or disseminated intravascular coagulation. Bone marrow aspirates and biopsies showed the same changes that were observed in patients treated with 250 mg/m^2.

At the lower dose levels, nausea and vomiting were not observed. However, at 250 mg/m^2, one patient developed 3+ vomiting and required hospitalization for replacement of fluids and electrolytes. Two other patients developed 1+ nausea at this dose level. At the 350-mg/m^2 dose level, three patients developed 1+ nausea and vomiting.

No objective regressions of tumor were noted during this trial, although the patient with the severe hyperglycemia did have stabilization of his progressive metastatic adenocarcinoma of the lung for 4+ months following his 250-mg/m^2 dose of TCN-P.

DISCUSSION

This phase I study of TCN-P evaluated a high-dose intermittent schedule, a potentially convenient schedule that was therapeutically active in preclinical testing. Toxicology studies suggested that with this schedule myelosuppression would be observed along with hepatic dysfunction, nausea, vomiting, and diarrhea.

Our results demonstrate that such a schedule is impractical for further clinical testing. Although one patient experienced only minimal toxicity at a dose of 350 mg/m^2, two patients treated with this dose developed progressive liver dysfunction. Even at the next lower dose, most patients experienced liver enzyme elevation, which was not completely reversible, as well as other metabolic abnormalities. The available data do not indicate whether these abnormalities are cumulative or dose-limiting. It is therefore difficult to define a safe dose for further evaluation.

The pathogenesis of the observed toxic effects is not obvious. The hyperglycemia was associated with inappropriately low insulin levels and therefore appeared to be related to either inhibition of insulin synthesis or interference with its release. This may be related to direct effects on protein synthesis or to interference with the effects of another nucleoside phosphate, cyclic-AMP. Changes in cyclic-AMP metabolism might also explain the abnormalities encountered in serum calcium and phosphorus concentrations. Furthermore, glucose intolerance as a result of peripheral resistance has been associated with hypophosphatemia (11); this, too, may have played a role in our patients.

The mechanism of the liver damage has also not been clearly defined. Many drugs, such as fluoxymesterone and prochlorperazine, can produce similar damage. Pharmacologic studies of TCN in beagle dogs demonstrated that most of the administered dose was excreted in the bile in such high concentration that the drug actually crystallized in the bile ducts (12). Recently, it has been demonstrated that after administration of TCN-P, rabbits excrete approximately 30% of the dose in bile as TCN.[5] Although no crystals were seen within the plugged bile canaliculi of our patients, TCN crystals may have formed early and then dissolved either spontaneously or during preparation of the pathologic slides. Whether this is related to the actual hepatic injury encountered is uncertain.

Phase II trials using this schedule cannot be recommended. Perhaps a different schedule, one which produces toxicity that is reversible and more predictable, will be more suitable for further clinical study.

REFERENCES

1. Schram KH, and Townsend LB. The synthesis of 6-amino-4-methyl-8-(β-D-ribofuranosyl) (4-H, 8-H) pyrrolo-[4,3,2-de] pyrimido[4,5-c] pyridazine, a new tricyclic nucleoside. Tetrahedron Lett 49:4757–4760, 1971.
2. Schweinsberg PD, Taylor HG, and Loo TL. Uptake and metabolism of the antitumor tricyclic nucleoside (TCN) by human red blood cells. Proc Am Assoc Cancer Res and ASCO 20:168, 1979.
3. Paterson ARP, Yang S, Lau EY , et al. Low specificity of the nucleoside transport mechanism of RPMI 1640 cells. Mol Pharmacol 16:900–908, 1979.
4. Plagemann PGW. Transport, phosphorylation, and toxicity of a tricyclic nucleoside in cultured Novikoff rat hematoma cells and other cell lines and release of its monophosphate by the cells. JNCI 57:1283–1295, 1976.
5. Bennett LL, Jr, Smithers D, Hill DL, et al. Biochemical properties of the nucleoside of 3-amino-1,5-dihydro-5-methyl-1,4,5,6,8-pentaazaazacenaphthylene (NSC-154020). Biochem Pharmacol 27:233–241, 1978.
6. Smith RG, Chan JC, and Loo TL. The in vitro oxidation of 3-amino-1,5-dihydro-5-methyl-1-β-D-ribofuranosyl-1,4,5,6,8-pentaazaacenaphthylene (TCN, NSC-154020). Proc Am Assoc Cancer Res and ASCO 21:20, 1980.
7. Roti Roti LW, Roti Roti JL, and Townsend LB. Studies on the mechanism of cytotoxicity of the tricyclic nucleoside NSC-154020 in L1210 cells. Proc Am Assoc Cancer Res and ASCO 19:40, 1978.
8. Townsend LB, Bhat GA, Chung F, et al. Synthesis, chemical reactivity, biological and chemotherapeutic activity of certain tricyclic nucleosides. INSERM 81:377–382, 1978.
9. Worting LL, Townsend LB, Crabtree GW, et al. A possible role for ecto-5′-nucleotidase in cytotoxicity and intracellular nucleotide formation from the tricyclic nucleoside-5′-phosphate (TCN-P). Proc Am Assoc Cancer Res and ASCO 22:257, 1981.
10. Clinical brochure. Tricyclic nucleoside 5′-phosphate. Bethesda, MD, National Cancer Institute, June 1981.
11. Defronso RA, and Lang R. Hypophosphatemia and glucose intolerance: evidence for tissue insensitivity to insulin. N Engl J Med 303:1259–1263, 1980.
12. Friedman J, Raulston GL, Furlong NB, et al. Disposition of 3-amino-5-methyl-1,5-dihydro-1-β-D-ribofuranosyl-1,4,5,6,8-pentaazaacenaphthylene (TCN, NSC-154020) in beagle dogs. Proc Am Assoc Cancer Res and ASCO 18:721, 1977.

[5]Powis G. Meeting of the Phase I Working Group, National Cancer Institute, Bethesda, MD, July 1982.

Correlation of Cellular Tritiated Thymidine Incorporation With Soft Agar Clonogenicity in Chemosensitivity Testing of Human Neuroblastoma Cells[1]

Gloria E. Johnson and Daniel L. Glaubiger[2,3,*]

A number of in vitro chemosensitivity tests for human tumors have been described recently (1–4), with much of the renewed interest based on the work of Salmon et al (5) demonstrating correlation of results obtained using a double-layer soft agar plating method and in vivo chemotherapeutic results. Their method is based upon the effects of chemotherapeutic agents on growth (number) of colony-forming cells. Other assay methods make use of quantitative morphologic or biochemical changes occurring in cells after exposure to cytotoxic agents (6,7) or use relative incorporation of radiolabeled metabolic precursors (4) to measure changes in cell metabolism following drug exposure.

In screening prospective new anticancer compounds, the double-layer soft agar method offers both advantages and disadvantages. Its main advantages are the use of primary tumor explants as a source of material and the demonstrated correlations with in vivo responses. The disadvantages include a number of technical difficulties related to sample preparation and processing, the low plating efficiencies of many tumor types, and the problems associated with long assay times (2–3 weeks) and visual counting methods. We have used human neuroblastoma cell lines as a model system to compare drug sensitivity curves obtained using relative incorporation of tritiated thymidine to those obtained using the soft agar cloning method. Curves were measured both for known chemotherapeutic agents and for new drugs. Data for new agents were obtained without knowledge of the agent's structure or the results of prior testing.

METHODS

Neuroblastoma cell lines were obtained from Children's Hospital of Philadelphia (CHP 100 and CHP 126), St. Jude Children's Research Hospital, Memphis, TN (EW), and from a patient seen in the Pediatric Oncology Branch of the National Cancer Institute (NCI), Bethesda, MD (KO). Cells were maintained in monolayer culture in RPMI-1640 medium (Gibco) supplemented with 10% non-heat-inactivated fetal bovine serum (Gibco). Immediately prior to drug exposure, cells were rinsed with 0.25% trypsin in Hanks' balanced salt solution (BSS) (Gibco) to obtain a cell suspension. Cell viability was then determined by trypan blue dye exclusion. Cells were counted visually using a standard hemacytometer and were suspended in Hanks' BSS to a concentration of 1.2×10^6 viable cells/ml.

Drug samples were obtained from the Developmental Therapeutics Program, Division of Cancer Treatment, NCI. Drugs were dissolved in steroid suspending vehicle (Division of Cancer Treatment, NCI), absolute alcohol, or normal saline, and were stored at –70°C. Drug samples were diluted with Hanks' BSS so that concentrations in 1.5-ml final volumes were as shown in the Results section. One milliliter of drug solution and 0.5 ml of cell suspension were mixed for each sample. Samples were then incubated for 1 hour at 37°C in a humidified, CO_2 (8%) incubator. Extracellular drug was then removed by washing twice with 3 ml of Hanks' BSS. The final supernatant was aspirated; the remaining cell pellet was resuspended in 1 ml of Hanks' BSS and was further diluted in growth media.

Samples to be assayed by relative incorporation of tritiated thymidine ([^{3}H]TdR) were handled as follows subsequent to drug exposure. A 0.1-ml aliquot of cell suspension (2×10^3 viable cells) in RPMI-1640, supplemented with 10% fetal bovine serum, was added to each of three wells of a 96-well, flat-bottom cluster plate. Cultures were then incubated until nontreated cells had grown to confluence (3–4 days) at 37°C in a humidified

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(98%) CO_2 (8%) atmosphere. [^{3}H]TdR (0.5 Ci; specific activity, 6.7 Ci/mmol [New England Nuclear Co, Boston, MA]) was then added to each well.

Eighteen hours later, 0.1 ml of 2.5% trypsin was added to each well and incubated for 15 minutes at 37°C to detach the cells, which grow in monolayer. Incubation and labeling times for these cell lines were chosen to coincide with conditions previously determined for optimum labeling of primary tumor explants for measurements of chemosensitivity (8). Using a multiple automatic sample harvester (Microbiological Associates, Bethesda, MD), the entire well volume (9) was then aspirated onto microfiber filter paper (Whatman Glass Co, Clifton, NJ; grade 934-AH), with continuous washing with distilled H_2O to remove extracellular [^{3}H]TdR label. The filter paper sections were placed in 12.5 ml of scintillation fluid (Hydrofluor, National Diagnostics, Somerville, NJ) and were counted in a Packard Tri Carb scintillation counter, model 3390 (Packard Instrument Co, Downers Grove, IL). Values obtained from triplicate samples were averaged and results were expressed as relative incorporation of [^{3}H]TdR into drug-treated cells compared with incorporation into control samples. [^{3}H]TdR incorporation averaged about 20,000 counts/minute (cpm) in control samples, with a low value of 4000 cpm.

Aliquots of drug-treated samples and control samples were also grown in a double-layer soft agar system initially described by Park et al (10) and subsequently modified by Hamburger and Salmon (11). Some modifications of the media were made as previously described. Bottom layers were made from 3% agar (Difco Bacto Agar, Detroit, MI) diluted one to six (for final concentration of 0.5% agar) with McCoy's 5A medium (Gibco) supplemented with 10% fetal bovine serum, 10% horse serum (Gibco), and a variety of nutrients as described by Pike and Robinson (12). Immediately before use, 10 ml of 3% tryptic soy broth (National Institutes of Health Media Unit), 0.6 ml of asparagine (0.6 mg/ml, Gibco), and 0.3 ml of DEAE-dextran (0.3 mg/ml, Pharmacia Fine Chemicals) were added to 40 ml of enriched medium. One milliliter of the mixture was placed in a 35-mm diameter sterile tissue culture dish and allowed to set. Top layers were made from 3% agar diluted one to ten (for a final concentration of 0.3% agar) with CMRL 1066 medium (Gibco) supplemented with 20% horse serum, 2% L-glutamine (200 mM, Gibco), $CaCl_2$ (4 mM), 2 units/ml of insulin (100 units/ml, Eli Lilly Co), L-ascorbic acid (vitamin C, 0.3 mM, Gibco), asparagine (0.6 mg/ml), and DEAE-dextran (0.3 mg/ml). Viable cells (2×10^4) were mixed with each milliliter of the above medium and 1 ml was then added to each culture plate. Aliquots of the resuspended cell mixture were examined to ensure that a single-cell suspension was obtained. Culture plates were evaluated for colony growth at 21 days after plating, either visually (when there

TABLE 1.—Compounds tested

Compound	NSC No.
Cisplatin	119875
Lomustine	79037
Chloroethyl nitrosourea (CNU)	47547
Doxorubicin	123127
Tricyclic nucleotide	280594
2-Fluoro ARA-AMP	328002
7-*O*-Methylnogarol	269148
Isopropyl pyrrolizine derivative	278214
6:β-Cyclodextrin	314334

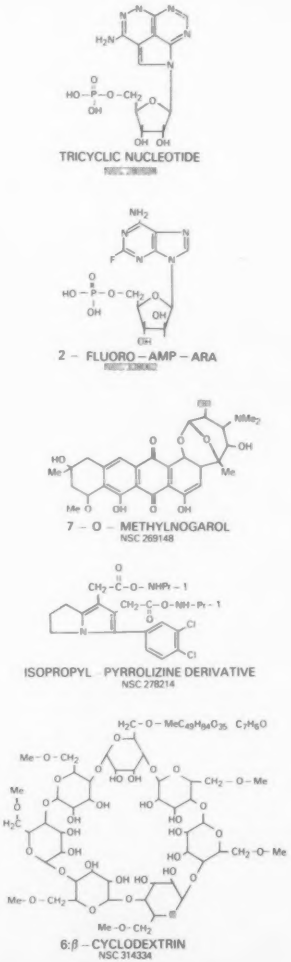

FIGURE 1.—Structures of tricyclic nucleotide, 2-fluoro ARA-AMP, 7-*O*-methylnogarol, isopropyl pyrrolizine derivative, and 6:β-cyclodextrin.

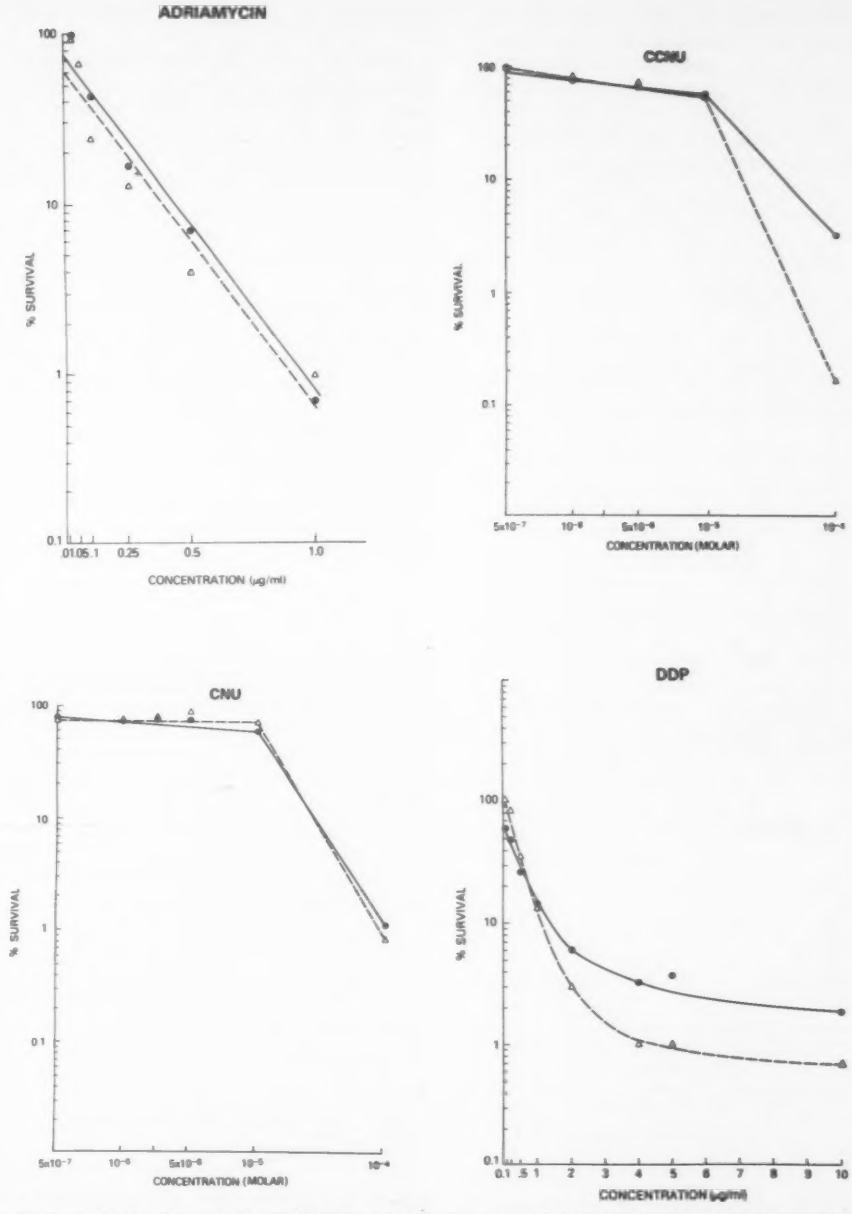

FIGURE 2.—Relative inhibition of growth of neuroblastoma cells following exposure to doxorubicin (Adriamycin), lomustine (CCNU), CNU, and cisplatin (DDP) for 1 hr at 37°C, as a function of drug concentration. Cell growth was monitored by [^{3}H]TdR incorporation (solid lines) and by colony-forming ability using a double-layer soft agar technique (dashed lines). Results indicate that all of these drugs are active cytotoxic agents against human neuroblastoma cells but with differing dependence on drug concentration. Results using both methods of assessing cytotoxicity are similar for all 4 agents.

were < 300 colonies in control plates) or using an automated image analyzer (Omnicon FAS-II, Bausch and Lomb). Triplicate samples were prepared and colony counts were averaged. Results were expressed as the ratio of colony numbers in drug-treated samples to colony numbers in control samples. The average number of colonies in control samples was 600, with a low value of 150.

RESULTS

A list of compounds tested using both methods is shown in table 1. The first four compounds listed are known chemotherapeutic agents; the last five are agents currently being evaluated. The structures of the five new compounds being evaluated are shown in figure 1. Four cell lines (KO, CHP 126, EW, and CHP 100) were

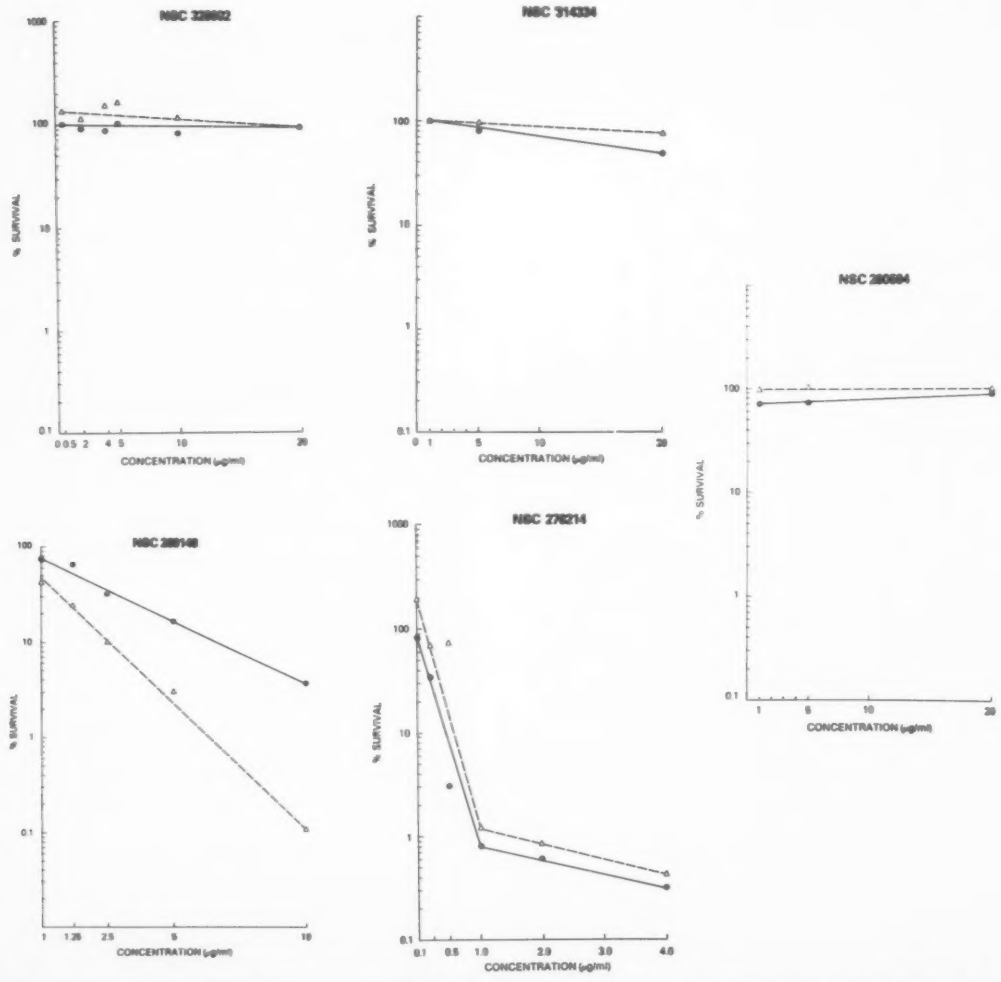

FIGURE 3.—Relative inhibition of growth of neuroblastoma cells following exposure to drugs for 1 hr at 37°C, as a function of drug concentration. Cell growth was monitored by [^{3}H]TdR incorporation (solid lines) and by colony-forming ability using a double-layer soft agar technique (dashed lines). The drugs shown are 2-fluoro ARA AMP (NSC-328002), 6:β-cyclodextrin (NSC-314334), 7-*O*-methylnogarol (NSC-269148), isopropyl pyrrolizine derivative (NSC-278214), and tricyclic nucleotide (NSC-280594). Results are similar for both techniques. No significant cytotoxicity (< 1 log cell kill) is noted for NSC-328002, NSC-314334, and NSC-280594. Significant cytotoxic effects (> 1 log cell kill) are noted using either method for NSC-269148 and NSC-278214.

used for each compound tested. Results were the same for all lines tested. Representative comparative data for each drug are shown in figures 2 and 3. Figure 2 shows the relative inhibition of cell growth subsequent to exposure to the four known chemotherapeutic agents, as measured by inhibition of colony formation in soft agar and by inhibition of [^{3}H]TdR incorporation. Similar results are obtained with both methods for all four drugs. Figure 3 shows results obtained for a series of new agents, again at various drug concentrations, using both methods. Similar curves are obtained for all compounds with both techniques.

The patterns of inhibition of cell growth and [^{3}H]TdR incorporation vary with the drug used. Agents exhibiting little or no response in terms of cytotoxicity have similar flat patterns of response as a function of drug dose. The drugs to which there is a cytotoxic response also show similar patterns, with the exception of the two nitrosourea compounds (CNU and lomustine), which both demonstrate an apparent lack of cytotoxicity at lower doses but are cytotoxic at higher doses. The other compounds which show cytotoxic effects against these human tumor cell lines do so beginning at the lowest dose level tested.

DISCUSSION

In vitro tests for chemotherapeutic activity can make significant contributions to an overall screening program for new agents by saving time and resources. Ideally, such tests should be rapid, reproducible, and readily transferable from laboratory to laboratory. The double-layer soft agar colony-forming assay adapted by Hamburger and Salmon (11) for use in many tumor explants has the attraction of selectively growing tumor cells from a wide variety of human neoplasms, often in numbers sufficient to permit in vitro testing of chemotherapeutic drugs. As has been demonstrated by several investigators, the in vivo correlation of negative in vitro results is extremely high (13–15), obviating exposure of patients to needless toxicity. The positive correlation between in vitro and in vivo results, while weaker, has allowed performance of in vitro phase II-type drug trials (16,17).

The results presented in this study show that, for new agents as well as established drugs, antitumor activity can be demonstrated by inhibition of incorporation of radiolabeled tracer into cellular DNA as well as by inhibition of colony formation in soft agar. The parallel results obtained in almost all cases suggest that the drug-induced inhibition of growth is similar for the sets of cells measured by each method. There is no drug among those tested to date which would be called inactive by one measure and active by the other. The advantages of using the soft agar cloning technique for screening compounds for possible antitumor activity are its rapidity, its quantitative reproducibility, and its use of reproducible source material. The disadvantages, again in terms of screening new compounds, are that it is not readily adaptable for use with individual tumor specimens and would not be suitable for the types of trials in which the soft agar method is presently applied. The present study is also incomplete in that it does not search for activity in various tumor types. However, availability of human tumor cell lines from different tissue types might permit more comprehensive screening of new agents. Both methods (incorporation of radiolabeled TdR and soft agar cloning) fail to distinguish agents that are cellular toxins without antitumor specificity from those drugs which have a therapeutic index, ie, drugs that have more effect against neoplastic cells than against normal cells. There is, to our knowledge, no in vitro test presently available that can make the distinction. Neither approach therefore is a substitute for in vivo tests on tumor-bearing animals in the evaluation of potential new anticancer drugs.

In summary, we have shown that the activities of four known chemotherapeutic agents and five new agents with chemotherapeutic potential, when measured against human neuroblastoma cell lines using inhibition of [^{3}H]TdR incorporation, correlate with results obtained for the same compounds using the double-layer soft agar colony-forming assay described by Hamburger and Salmon (11). In terms of screening new agents, a technique using cell lines and rapid measures of cytotoxicity offers a number of advantages, as mentioned above, but some disadvantages as well, particularly the lack of individualization of the test. The use of reproducible source material and the availability of tumor cells derived from several individuals and tissues may mitigate those disadvantages.

REFERENCES

1. Berry RJ, Laing AH, and Wells J. Fresh explant culture of human tumours in vitro and the assessment of sensitivity to cytotoxic chemotherapy. Br J Cancer 31:218–227, 1975.
2. Holmes HL, and Little JM. Tissue-culture microtest for predicting response of human cancer to chemotherapy. Lancet 2:985–987, 1974.
3. Roper PR, and Drewinko B. Comparison of in vitro methods to determine drug induced cell lethality. Cancer Res 36:2182–2188, 1976.
4. Shrivastav S, Bonar RA, Stone KR, et al. An in vitro assay procedure to test chemotherapeutic drugs on cells from human solid tumors. Cancer Res 40:4438–4442, 1980.
5. Salmon SE, Hamburger HW, Soehnlen B, et al. Quantitation of differential sensitivity of human tumor stem cells to anticancer drugs. N Engl J Med 298:1321–1327, 1978.
6. Wright JC, and Walker D. A predictive test for the selection of cancer chemotherapeutic agents for the treatment of human cancer. J Surg Oncol 7:381–393, 1975.

7. Weisenthal LM, and Marsden JM. A novel dye exclusion assay for predicting response to cancer chemotherapy. Proc Am Assoc Cancer Res and ASCO 22:155, 1981.
8. Friedman HM, and Glaubiger DL. Assessment of in vitro drug sensitivity of human tumor cells using [^{3}H] thymidine incorporation in a modified human tumor stem cell assay. Cancer Res, in press.
9. Hartzman RJ, Bach ML, Bach FH, et al. Precipitation of radioactively labeled samples: a semi-automatic multiple sample processor. Cell Immunol 4:182–186, 1972.
10. Park CH, Bergsagel DE, and McCullough EA. Mouse myeloma tumor stem cells: a primary cell culture assay. JNCI 46:411–422, 1971.
11. Hamburger AW, and Salmon SE. Primary bioassay of human tumor stem cells. Science 197:461–463, 1977.
12. Pike BL, and Robinson WA. Human bone marrow colony growth in agar-gel. J Cell Physiol 76:77–84, 1970.
13. Salmon SE, Soehnlen BJ, Durie BGM, et al. Clinical correlations of drug sensitivity in the tumor stem cell assay. Proc Am Assoc Cancer Res and ASCO 20:340, 1979.
14. Alberts DS, Salmon SE, Chen HSG, et al. Correlative and predictive accuracy of the human tumor stem cell assay for anticancer drug activity in ovarian cancer patients. Proc Am Assoc Cancer Res and ASCO 21:451, 1980.
15. Von Hoff DD. Clinical correlations of drug sensitivity in the tumor stem cell assay. Proc Am Assoc Cancer Res and ASCO 21:134, 1980.
16. Von Hoff DD, Coltman CH, Jr, and Forseth B. Activity of mitoxantrone in a human tumor cloning system. Cancer Res 41:1853, 1981.
17. Ahmann F, Meyskens F, Jones S, et al. A broad phase II trial of AMSA with in vitro stem cell culture drug sensitivity correlation. Proc Am Assoc Cancer Res and ASCO 21:369, 1980.

Plasma Platinum Levels: Relationship to Cisplatin Dose and Nephrotoxicity[1,2]

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Renal dysfunction is the dose-limiting toxic effect for many patients receiving cisplatin (CP). Despite hydration and/or forced diuresis, some patients develop nephrotoxicity, and patients at risk cannot be easily identified. We studied 77 patients with several types of carcinoma who received 115 cycles of CP, by 24-hour infusion, at doses of 40–100 mg/m². Blood samples for platinum (Pt) analysis were obtained during and after CP infusion; plasma Pt levels were dose- and time-dependent. A significant rise in serum creatinine was seen after the first cycle and cumulatively over subsequent CP cycles. The largest subgroup of patients, those who received 80 mg/m², was analyzed for predictors of nephrotoxicity. Twenty-five percent of patients exhibited nephrotoxicity (> 30% rise in serum creatinine or > 30% fall in creatinine clearance). These nephrotoxic patients had significantly higher plasma Pt levels during CP infusion than did nonnephrotoxic patients. Age, sex, cycle number, and pretreatment creatinine did not predict nephrotoxicity. Patients predisposed to nephrotoxicity with CP chemotherapy may be identifiable, on the basis of elevated plasma Pt, early in the course of CP infusion. [Cancer Treat Rep 67:169–172, 1983]

Cisplatin (CP), a relatively new chemotherapeutic agent, is active against a variety of solid tumors (1). Renal damage appears to be dose-limiting for a substantial proportion (10%–50%) of patients receiving the drug (1–3). Although nephrotoxicity may be transient, it is often permanent and occasionally fatal (3–5). There is some evidence that both the incidence and the severity of nephrotoxicity are dose-related (3,4).

Little is known about the mechanism of CP-induced nephrotoxicity (5–7). Factors that predispose patients to the development of nephrotoxicity have not been clearly identified, although inadequate hydration and concomitant aminoglycoside administration are suspect (5,8,9). It has been observed, in both animals and man, that maneuvers which increase urine flow around the time of CP administration appear to decrease the incidence and/or severity of CP nephrotoxicity (4,10,11). Plasma platinum (Pt) levels have been described in patients receiving two different doses of CP by rapid iv infusion (12) and in children following a 6-hour infusion (13). The utility of plasma Pt levels for predicting or monitoring nephrotoxicity has not been well-defined.

The purpose of this study was to determine whether Pt levels can predict for nephrotoxicity. In addition, we studied the effect of dose on plasma Pt levels at various times during and after a 24-hour infusion of CP, the change in serum creatinine values following CP administration, and other predictors of nephrotoxicity.

METHODS

Data were accumulated on 115 cycles of CP in 77 patients with head and neck, testicular, prostatic, lung, or ovarian cancer treated at Stanford University Hospital between July 1977 and May 1980. These patients received CP at doses of 40, 60, 80, or 100 mg/m² every 3 weeks. All of the CP was given by continuous infusion over 24 hours with iv hydration to maintain urine output > 150 ml/hour during and for 24 hours after the infusion (14). Seven patients received a single dose of furosemide (20 mg) during infusion.

Samples for plasma Pt levels were collected in heparinized tubes at 6, 12, 24, and 48 hours after initiation of CP infusion. Levels for all four time periods were not available for each patient in the study. Pt levels were determined 3 weeks after infusion for ten cycles. After collection, blood samples were centrifuged at 3000 rpm for 10 minutes in a table-top centrifuge to remove cells. Elemental Pt assay in plasma was measured by flameless atomic absorption spectrophotometry (15). No attempt was made to separate free from bound drug or to differentiate parent compound from metabolites. Thus, our results are expressed as total Pt. A serum creatinine determination was done prior to each cycle and 2–3 weeks after CP administration. Specimens were analyzed on a multichannel analyzer in the clinical laboratory.

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TABLE 1.—Plasma Pt levels during and after CP infusion: effect of dose

CP dose (mg/m^2)	Pt level (μg/ml)*			
	6 hrs	12 hrs	24 hrs	48 hrs
40	0.53 ± 0.08 (3)	0.81 ± 0.06 (9)	1.16 ± 0.10 (10)	1.05 ± 0.35 (2)
60	0.83 ± 0.12 (7)	1.09 ± 0.14 (13)	1.46 ± 0.15 (17)	0.84 ± 0.07 (3)
80	0.70 ± 0.02 (34)	1.08 ± 0.08 (25)	1.62 ± 0.06 (52)	1.32 ± 0.07 (33)
100	0.76 ± 0.07 (9)	1.34 ± 0.11 (21)	2.03 ± 0.12 (27)	1.73 ± 0.20 (13)
	NS	$P < 0.01$	$P < 0.01$	$P = 0.01$

*Values = mean ± SE. Nos. in parentheses = No. of Pt cycles. NS = not significant. *P* value represents 1-way analysis of variance for dose effect at each time.

The largest subgroup of patients, those who received a CP dose of 80 mg/m^2, was selected for more detailed analysis of "predictors" for nephrotoxicity. The numbers of patients in the other subgroups (40, 60, and 100 mg/m^2) were too small for meaningful statistical analysis. Forty patients received 57 cycles of CP at 80 mg/m^2. Besides the above-mentioned plasma Pt levels and serum creatinine determinations, 24-hour urine specimens for creatinine clearance were obtained prior to and 3 weeks following chemotherapy.

Nephrotoxicity was defined as a > 30% rise in serum creatinine and/or a > 30% fall in creatinine clearance after any cycle of CP. Nine cycles were eliminated from analysis because of unavailable posttreatment creatinine values. Nephrotoxicity was based on creatinine clearance data in 28 cycles and on serum creatinine values in 20 cycles.

Statistical analysis was performed on a Digital Equipment Corporation PDP-10 computer using the Statistical Package for the Social Sciences. Tests included analysis of variance and t test for paired samples.

RESULTS

Plasma Pt levels during and after a CP infusion were affected by CP dose (table 1). This effect was statistically significant at 12, 24, and 48 hours. The 12-hour Pt levels ranged from 0.81 ± 0.06 μg/ml for the 40-mg/m^2 dose to 1.34 ± 0.11 μg/ml for the 100-mg/m^2 dose ($P < 0.01$). For the same doses, peak Pt levels at 24 hours ranged from 1.16 ± 0.10 to 2.03 ± 0.12 μg/ml ($P < 0.01$), and 48-hour levels ranged from 1.05 ± 0.35 to 1.73 ± 0.20 μg/ml ($P = 0.01$). Detectable Pt levels persisted long after CP infusion. Following 14 cycles, Pt levels were 0.22 ± 0.11 μg/ml 3–4 weeks after infusion.

Administration of CP had small but reproducible effects on serum creatinine regardless of dose or cycle. Pre- and post-CP serum creatinine values for all patients in the study for the first and all cycles are presented in table 2. Statistically significant increases in serum creatinine occurred, apparently with any cycle of CP treatment, including the first. The change in serum creatinine for all cycles by dose is shown in table 3. Some change occurred even at relatively low doses of CP; creatinine increases were statistically significant at 60, 80, and 100 mg/m^2. Furthermore, these changes were cumulative when more than one cycle of CP was administered.

We attempted to identify factors which could predict nephrotoxicity with CP administration. Because we wished to examine the relationship of Pt levels to nephrotoxicity, and because Pt levels are dose-dependent, we chose the largest subgroup of patients, those who

TABLE 2.—Change in serum creatinine value with CP administration, by cycle

	Serum creatinine (mg/dl)*		
	Before CP	After CP	*P* value
1st cycle (n = 39)	0.94 ± 0.04	1.07 ± 0.05	< 0.01
All cycles (n = 103)	1.07 ± 0.04	1.20 ± 0.04	< 0.01

*Values = mean ± SE.

TABLE 3.—Change in serum creatinine value with CP administration, by dose (all cycles)

Dose (mg/m^2)	Serum creatinine (mg/dl)*		P value
	Pretreatment creatinine	Posttreatment creatinine	
40 (n = 10)	1.39 ± 0.17	1.50 ± 0.19	0.38
60 (n = 17)	1.25 ± 0.10	1.42 ± 0.12	< 0.01
80 (n = 48)	0.92 ± 0.03	1.08 ± 0.04	< 0.01
100 (n = 26)	1.07 ± 0.05	1.19 ± 0.06	< 0.01

*Values = mean ± SE.

received a dose of 80 mg/m^2, for this analysis. Nephrotoxicity, as defined by us, occurred in 12 of 48 (25%) CP cycles at this dose. There was no significant difference in age, sex, or cycle number between the patients without nephrotoxicity and those with nephrotoxicity (table 4). Patients who subsequently developed nephrotoxicity had a somewhat lower pretreatment creatinine (0.81 ± 0.04) than did those patients not developing nephrotoxicity (0.97 ± 0.04; $P < 0.05$). However, there was no difference in pretreatment creatinine clearance values between the two groups.

For the subgroup of patients who received 80 mg/m^2 of CP, plasma Pt levels during and shortly after infusion appeared to correlate with nephrotoxicity. Those patients who developed nephrotoxicity exhibited elevated Pt early in the course of a 24-hour infusion (fig 1); this elevation was apparent at 6 hours and was statistically significant at 12 hours. Patients developing nephrotoxicity had 12-hour levels of 1.31 ± 0.05 μg/ml, while those who did not develop nephrotoxicity had 12-hour levels of 0.97 ± 0.10 μg/ml ($P < 0.05$). Pt remained elevated in the toxicity group at 24 hours: patients who developed toxicity had levels of 1.79 ± 0.15 μg/ml; those who did not had levels of 1.52 ± 0.07 ($P < 0.05$). Subtraction of the 48-hour Pt level from the 24-hour Pt level yields a crude estimate of Pt excretion over the first 24 hours after Pt infusion. The difference between 24- and 48-hour levels was 0.12 ± 0.12 μg/ml for the group with toxicity and 0.28 ± 0.04 μg/ml for the group with no toxicity ($0.10 > P > 0.05$).

DISCUSSION

We have demonstrated that plasma Pt levels rise and fall in predictable fashion during and after a 24-hour infusion of CP. Furthermore, Pt levels are dose-dependent at any given time point during or after CP infusion. Most of the patients who received CP manifested some degree of renal dysfunction, as evidenced by a rise in serum creatinine. This phenomenon is not dependent on prior exposure to CP; it occurs with the first cycle of drug administration. We were unable to demonstrate any dose dependence of renal dysfunction, but we used a maximum CP dose of only 100 mg/m^2. Most patients received a dose of 80 mg/m^2, all by 24-hour infusion.

For the largest subgroup, those who received 80 mg/m^2, there was a correlation between plasma Pt level at 12 and 24 hours and the subsequent development of nephrotoxicity. Although none of our patients developed CP-induced renal failure, there was a > 30% rise in serum creatinine or a > 30% fall in creatinine clearance following 25% of CP cycles at 80 mg/m^2. Other authors have suggested that inadequate hydration and/or concurrent aminoglycoside administration may enhance the nephrotoxicity of CP (5,8,9); none of our patients was clinically dehydrated, nor did any receive other nephrotoxic drugs concurrently. In our patients, predisposition to the development of CP nephrotoxicity did not correlate with age, sex, pretreatment creatinine clearance, or number of previous cycles of CP. The num-

TABLE 4.—Nephrotoxicity: predisposing factors (dose = 80 mg/m^2)

	No toxicity (n = 36)	Toxicity (n = 12)	Significance*
Age in yrs	50.1	49.1	NS
Cycle No.	2.1	2.6	NS
Pretreatment creatinine (mg/dl)	0.96	0.81	$P < 0.05$
Pretreatment creatinine clearance (ml/min)	85.8†	84.5‡	NS

*NS = not significant.
†n = 30.
‡n = 11.

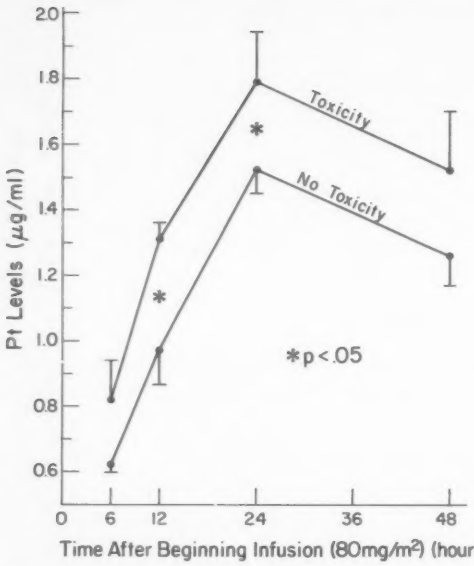

FIGURE 1.—Correlation of Pt levels with nephrotoxicity. Dose of CP was 80 mg/m². Nephrotoxicity was defined as a > 30% rise in serum creatinine or a > 30% fall in creatinine clearance.

ber of patients with hydronephrosis, abnormal urinalysis, abnormal hepatic function, or third-space fluid and the number who received furosemide were too small for correlation.

Patients in this study who developed nephrotoxicity had elevated Pt early in the course of a 24-hour infusion. Furthermore, Pt excretion during the first 24 hours after CP infusion (as estimated by the difference between 24- and 48-hour levels) may be impaired in the group subsequently manifesting nephrotoxicity. These data imply that some factor present prior to drug administration or some event early in the course of infusion is responsible for a greater than average rise in serum creatinine with CP administration in this subgroup of patients. Several alternative explanations exist. First, elevated Pt might cause nephrotoxicity. Such an effect might be due to CP itself or its metabolites (16). Second, elevated Pt may reflect renal damage occurring early in the course of an infusion, ie, prior to 6 hours. Third, other factors may predispose patients to nephrotoxicity and cause elevated Pt. Such factors might include differences in pretreatment hydration status or individual differences in CP distribution, metabolism, or excretion. Even though the cause for elevated Pt in these patients is uncertain, it does appear that patients who subsequently develop CP nephrotoxicity may be identifiable on the basis of elevated plasma Pt early in the course of an infusion. It will be of interest to see whether dose attenuation during CP infusion in these patients will lessen the probability of subsequent nephrotoxicity.

REFERENCES

1. LIPPMAN AJ, HELSON C, HELSON L, ET AL. Clinical trials of *cis*-diamminedichloroplatinum (NSC-119875). Cancer Chemother Rep 57:191–200, 1973.
2. OSTROW S, EGORIN MJ, HAHN D, ET AL. High-dose cisplatin therapy using mannitol versus furosemide diuresis: comparative pharmacokinetics and toxicity. Cancer Treat Rep 65:73–78, 1981.
3. ROSSOF AH, SLAYTON RE, and PERLIN CP. Preliminary clinical experience with *cis*-diamminedichloroplatinum (II) (NSC-119875, CACP). Cancer 30:1451–1456, 1972.
4. COMIS RL. Cisplatin nephrotoxicity: the effect of dose, schedule, and hydration scheme. *In* Cisplatin, Current Status and New Developments (Prestayko AW, Crooke ST, and Carter SK, eds). New York, Academic Press, 1980, pp 485–494.
5. DENTINO M, LUFT FC, YUM MN, ET AL. Long-term effects of *cis*-diamminedichloride platinum (CDDP) on renal function and structure in man. Cancer 41:1274–1281, 1978.
6. GONZALEZ-VITALE JC, HAYES DM, CVITKOVIC E, ET AL. The renal pathology in clinical trials of *cis*-platinum (II) diamminedichloride. Cancer 39:1362–1371, 1977.
7. LEVI J, JACOBS C, KALMAN SM, ET AL. Mechanism of *cis*-platinum nephrotoxicity. I. Effects of sulfhydryl groups in rat kidneys. J Pharmacol Exp Ther 213:545–550, 1980.
8. RANDOLPH VL, and WITTES RE. Weekly administration of *cis*-dichlorodiammineplatinum (II) without hydration or osmotic diuresis. Eur J Cancer 14:753–756, 1978.
9. GONZALEZ-VITALE JC, HAYES DM, CVITKOVIC E, ET AL. Acute renal failure after *cis*-dichlorodiammineplatinum(II) and gentamicin-cephalothin therapies. Cancer Treat Rep 62:693–698, 1978.
10. CVITKOVIC E, SPAULDING J, BETHUNE V, ET AL. Improvement of *cis*-dichlorodiammineplatinum (NSC-119875): therapeutic index in an animal model. Cancer 39:1357–1361, 1977.
11. HAYES DM, CVITKOVIC E, GOLBEY RB, ET AL. High dose *cis*-platinum diammine chloride: amelioration of renal toxicity by mannitol diuresis. Cancer 39:1372–1381, 1977.
12. HIMMELSTEIN KJ, PATTON TF, BELT RJ, ET AL. Clinical kinetics of intact cisplatin and some related species. Clin Pharmacol Ther 29:658–664, 1981.
13. CROM WR, EVANS WE, PRATT CB, ET AL. Cisplatin disposition in children and adolescents with cancer. Cancer Chemother Pharmacol 6:95–99, 1981.
14. JACOBS C, BERTINO JR, GOFFINET DR, ET AL. 24-Hour infusion of *cis*-platinum in head and neck cancers. Cancer 42:2135–2140, 1978.
15. LEROY AF, WEHLING ML, SPONSELLER HC, ET AL. Analysis of platinum in biological materials by flameless atomic absorption spectrophotometry. Biochem Med 18:184–191, 1977.
16. JACOBS C, KALMAN SM, TRETTON M, ET AL. Renal handling of *cis*-diamminedichloroplatinum(II). Cancer Treat Rep 64:1223–1226, 1980.

Activity of Two Phase I Drugs, Homoharringtonine and Tricyclic Nucleotide, Against Surgical Explants of Human Tumors in the 6-Day Subrenal Capsule Assay[1,2]

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The potential clinical activity of new chemotherapeutic agents still undergoing clinical toxicology trials was examined by testing the phase I drugs homoharringtonine (HHT) and tricyclic nucleotide (TCNP) against fresh surgical explants of human tumors in the 6-day in vivo subrenal capsule assay. Eighty tumors representing different histologic types including breast, lung, colon, ovarian, and cervical, as well as neoplasms of undiagnosed origin, were screened simultaneously against HHT, TCNP, and five standard chemotherapeutic agents used clinically for treatment of the specific type of cancer. Thus, activity of HHT and TCNP could be compared directly with that of standard agents tested against a spectrum of tumor types. Treatment schedule was daily on Days 1–5, and the criterion for drug activity was > 20% regression of tumor grafts. HHT was active against 13 of 80 tumors, with a response rate of 16%, and resulted in an average tumor size reduction of 38%. TCNP, which was active against 36 of 80 tumors, yielded a response rate of 45% and resulted in an average of 45% reduction in tumor size. The response rate to TCNP was nearly threefold greater than that to HHT, and the average percent tumor reduction was substantially greater; this indicated a superior degree of antitumor activity for TCNP. TCNP was either more active or comparable in activity to the standard agents used to treat a particular tumor type, comparing favorably with cyclophosphamide. Thirty-two of 80 tumors (40%) were unresponsive to all drugs tested, one was responsive only to HHT and TCNP, one only to HHT, and four only to TCNP. Dose responses to both drugs were obtained with sensitive tumors. Although the relative activity between new chemotherapeutic agents and clinically active drugs against human tumors has been demonstrated using the 6-day subrenal capsule assay, the validity of the assay in predicting the potential clinical activity of new chemotherapeutic agents will ultimately depend on the results of phase II trials. [Cancer Treat Rep 67:173–178, 1983]

The subrenal capsule (SRC) assay was initially developed as a rapid in vivo method to screen drugs for anticancer activity against a panel of human tumors established in serial transplantation in the immunodeficient nude athymic mouse (1). This human tumor-nude mouse model permits completion of drug assays in 11 days (2) in contrast to assays in which tumor fragments are implanted sc and require several weeks before test results are available. The SRC methodology has also been applied to screening of drugs against fresh surgical explants of human tumors as first transplant generation grafts using the normal immunocompetent mouse as the

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xenograft host in a 6-day assay (3). In initial studies in which fresh surgical explants of human breast tumors were tested in this assay, not only were clinically active agents detected but also differences in sensitivity of the various breast tumors to the same agents yielded response rates indicative of the relative clinical potential of each drug for the specific treatment of breast cancer (4).

In view of these findings, the present study was undertaken to screen new chemotherapeutic agents while in phase I toxicology trials to determine potential clinical activity. Two phase I drugs, homoharringtonine (HHT), isolated from an evergreen plant native to the mainland of China (5), and tricyclic nucleotide (TCNP), a tricyclic nucleoside 5′-phosphate ester (6), were tested against fresh surgical explants of human tumors of different histologic types using the 6-day SRC assay. Standard chemotherapeutic agents used clinically to treat specific histologic types of cancer were tested simultaneously against each tumor specimen so that direct comparisons could be made between activity of the phase I drugs and standard agents. Should superior activity among phase I drugs be detected, decision-aiding priorities could be established for entry of a new agent into phase II trials.

MATERIALS AND METHODS

The methodology for the SRC assay has been described in a previous publication (1). The technique permits in situ measurement of a tumor fragment under the renal capsule at the time of implant and a second measurement on the final experiment day. Hence, a change in tumor size can be determined and utilized as the parameter to evaluate tumor responsiveness in a manner analogous to the clinical evaluation of regressive and progressive disease.

Human tumor tissue was obtained from hospitals immediately after the patients' surgery and transported to the laboratory in cell culture medium 199. Assays were run on tumor specimens in the sequence received with no intentional selection regarding tumor type or patient clinical history. The various histologic types of tumors received and numbers in each category are listed in table 1.

Upon receipt, the tissue was cut into 1 × 1 × 1-mm pieces, and a single piece was implanted under the renal capsule of each of 43 normal immunocompetent (CDF_1 hybrid) mice. An initial in situ measurement was taken of two diameters (length and width) of each fragment using a stereoscopic microscope equipped with an ocular micrometer and the magnification calibrated at 10 omu/mm. Measurements were converted to tumor size in omu expressed as the average of the two diameters, (length + width)/2, for each graft. After implantation, animals were randomized into test (five mice) and control (eight mice) groups. Each assay included the two phase I drugs in addition to five chemotherapeutic agents used in the clinical treatment of the particular histologic type of tumor.

TABLE 1.—Tumor types screened against HHT and TCNP in 6-day SRC assays

Type	No.
Breast	19
Lung	12
Ovarian	10
Colorectal	8
Cervical	8
Small bowel	2
Gastric	2
Endometrial	3
Leukemia	1
Lymphoma	4
Renal	4
Pancreatic	1
Unknown	6
Total	80

Twenty different clinical agents were tested in this study. The drugs most commonly tested in parallel with HHT and TCNP were methotrexate, 5-FU, cyclophosphamide, cisplatin, and doxorubicin. Dose levels, routes of administration, and treatment schedules are given in table 2. Doses were selected on the basis of toxicity and antitumor effect in rodent systems, ie, effectiveness without deaths or excessive body weight loss during the assay time frame. The final body weight/initial body weight (FBW/IBW) ratio for each drug listed in table 2 was used as an indicator of systemic toxicity. An FBW/IBW ratio < 0.79 was considered to reflect toxic body weight loss. The same dose levels, route of administration, and schedule of treatment were used for a specific drug in each experiment. Thus, differences in responsiveness among different tumor specimens reflect variations in the drug sensitivity of a particular tumor rather than differences due to dose or treatment schedule.

Since tumors were assayed in the sequence received at the laboratory, there was no intentional selection of tumors by histologic type. There were, however, sufficiently large numbers of breast, lung, ovarian, colorectal, and cervical tumors (table 1) to permit direct comparison of the activity of HHT and TCNP with standard agents used in the clinical treatment of these particular types of cancer. These agents are listed in table 3; dose levels were those provided in table 2.

Drug treatment was initiated the day following tumor implantation. HHT was prepared in saline and administered iv at a dose of 2.0 mg/kg/injection, daily on Days 1–5; TCNP was formulated in water and administered sc at a dose of 50 mg/kg/injection, daily on Days 1–5. Dose levels for these two compounds were based on

TABLE 2.—Dose levels and routes of administration for standard chemotherapeutic agents tested in 6-day SRC assays

Common name	Abbreviation	Dose level (mg/kg/injection)*	Route	FBW/IBW† ratio
Methotrexate	MTX	4.0	sc	0.87
Thiotepa	TT	6.0	sc	0.88
Melphalan	L-PAM	6.0	sc	0.84
5-FU	5-FU	50.0	sc	0.84
Cyclophosphamide	CTX	50.0	sc	0.87
Mitomycin	Mit-C	0.7	sc	0.97
Vincristine	VCR	1.0	sc	0.83
Streptozocin	SZC	100.0	sc	0.92
Cisplatin	*cis*-DDP	2.0	sc	0.86
Doxorubicin	ADR	4.0	iv	0.86
Bleomycin	BLEO	30.0	sc	0.83

*Dose schedules: all drugs except SZC were administered daily on Days 1–5; SZC was administered every 2 days × 3 (Days 1, 3, and 5).

†Final body weight on Day 6/initial body weight on Day 0.

historical drug screening and toxicity data obtained from the Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). Body weight changes were measured during the course of each assay to monitor relative systemic drug toxicity and are expressed as the mean FBW/IBW ratio.

On Day 6 (1 day after the last treatment), animals were killed and a final in situ tumor measurement was recorded. The change in tumor size (ΔTS value) was calculated for each animal, and the average ΔTS values were computed for test and control groups. The criterion designated for drug activity is regression from initial graft size giving an average ΔTS value < -1.0 omu, which is equivalent to $> 20\%$ regression based on tumor weight. The average ΔTS value for the control group is not included in calculation of antitumor activity, but serves specifically as a control indicator of the quality and viability of tumor tissue utilized for implantation of the assay. Assays were considered evaluable if xenografts in the control group remained stable in size (mean ΔTS ± SD: 0.0 ± 0.5 omu) or grew over the 6-day assay period giving a mean ΔTS ≥ -0.5 omu.

The CDF_1 (BALB/c × DBA/2) mice used as test hosts were provided by the Mammalian Genetics and Animal Production Section of the Division of Cancer Treatment, National Cancer Institute. Compounds used in these studies were supplied under the auspices of the Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment.

RESULTS

A total of 88 tumors representing diverse histologic types including breast, lung, ovarian, colon, cervical, renal, and lymphoma were screened against HHT and TCNP in this study. Of the 88 experiments carried out, eight exhibited unsatisfactory control growth and were therefore considered invalid, providing a 90% evaluable assay rate. These eight experiments were eliminated from analysis. This percentage of evaluable assays is comparable to that reported previously in studies in which chemotherapeutic agents were screened against fresh surgical explants of human breast tumors in the 6-day SRC assay (4).

A dose effect on growth of first transplant generation grafts was exhibited by both HHT and TCNP against responsive human tumors (table 4). Over the dose ranges tested, acute toxicity was observed at the highest dose for each drug, activity (tumor regression) at the intermediate dose, and inactivity at the lowest dose. Slight to moderate body weight losses were observed for both HHT and TCNP at doses which were inactive or active. Toxicity-activity data from such dose response assays were used in conjunction with historical anticancer drug

TABLE 3.—Standard agents tested against different histologic types of human tumors in 6-day SRC assays

Tumor type	Drugs tested				
Breast	CTX	ADR	MTX	5-FU	L-PAM
Lung	CTX	ADR	MTX	*cis*-DDP	BLEO
Ovarian	CTX	ADR	5-FU	*cis*-DDP	TT
Colorectal	CTX	5-FU	VCR	Mit-C	SZC
Cervical	CTX	ADR	MTX	*cis*-DDP	VCR

TABLE 4.—Dose-response effects for toxicity and activity of HHT and TCNP in the 6-day SRC assay

Phase I agent	Cervical tumor assay			
	Dose (mg/kg/injection)	ΔTS in omu (mean ± SE)*	FBW/IBW ratio	No. of deaths/ total
HHT	4.0	Toxic†	—	5/5
	2.0	−1.4 ± 1.0‡	0.92	0/6
	1.0	−0.4 ± 0.3§	0.92	0/5
TCNP	100	Toxic	—	2/5
	50	−1.5 ± 0.7‡	0.81	0/5
	25	−0.6 ± 0.3‡	0.90	0/4
Control	—	+0.6 ± 0.3	0.95	0/11

*Criterion for activity: average ΔTS < −1.0 omu between Days 0 and 6.
†Doses at which there were ≥ 35% deaths were considered toxic.
‡Test results significant from the control at the $P < 0.01$ level.
§Test results significant from the control at the $P < 0.05$ level.

screening and toxicity data for conventional mice, as well as with data from transplantation-established human tumor-nude mouse models, in selecting doses for testing HHT and TCNP which would be sufficiently high to elicit antitumor activity without excessive drug toxicity.

Table 5 illustrates the chemotherapy response of a lung and an ovarian tumor to HHT, TCNP, and five chemotherapeutic agents used clinically for treatment of these particular histologic types. With the criterion for drug activity designated as a ΔTS value < −1.0 omu, the compounds have been ranked in order of activity against each tumor, from the most active at the top to the least active at the bottom. Therefore, the larger the numerical value of the −ΔTS, the more active the drug. The compounds have also been subdivided within this ranking into active and inactive groups for each assay. As can be seen from these examples, ranking of drugs in this manner provides an activity profile which permits evaluation of the sensitivity of a particular human tumor to a spectrum of drugs, as well as comparison of activity of specific drugs against different histologic types of tumors.

A summary of results of screening HHT and TCNP for activity against surgical explants of human tumors in the 6-day SRC assay is given in table 6. Results for the standard agents most commonly assayed against the same spectrum of tumors have been included for comparison. The data were evaluated using the clinically relevant parameters, response rate and percent tumor reduction.

HHT was active against 13 of 80 tumors, with a response rate of 16%, and resulted in an average tumor size reduction of 38%. TCNP, which was active against 36 of 80 tumors, yielded a response rate of 45% and resulted in an average of 45% reduction in tumor size (table 6). The response rate of TCNP was nearly threefold greater than that of HHT and the average percent tumor reduction was substantially greater, indicating a superior degree of antitumor activity for TCNP. The

TABLE 5.—Drug response profiles of human tumors as 1st transplant generation grafts in 6-day SRC assays

Lung		Ovarian	
Agent	ΔTS in omu (mean ± SE)*	Agent	ΔTS in omu (mean ± SE)*
TCNP	−4.4 ± 0.2†	TCNP	−2.9 ± 0.8‡
CTX	−3.1 ± 0.5†	TT	−2.3 ± 0.6‡
MTX	−2.1 ± 0.2†	5-FU	−1.7 ± 0.5‡
HHT	−1.5 ± 0.6‡		
ADR	−1.0 ± 0.6‡	CTX	−0.9 ± 0.3‡
cis-DDP	+0.3 ± 0.6	HHT	−0.3 ± 1.2
BLEO	+0.9 ± 0.5	ADR	0.0 ± 0.6§
		cis-DDP	0.0 ± 0.3
Control	+1.0 ± 0.4	Control	+2.4 ± 0.8

*Criterion for activity: average ΔTS < −1.0 omu between Days 0 and 6.
†Test results significant from the control at the $P < 0.001$ level.
‡Test results significant from the control at the $P < 0.01$ level.
§Test results significant from the control at the $P < 0.05$ level.

TABLE 6.—Comparison of activity of HHT and TCNP with standard agents tested against fresh surgical explants of human tumors in 6-day SRC assays

Drug	No. of tumors responsive/ No. tested*	% response	Average % regression
TCNP	36/80	45	45
HHT	13/80	16	38
CTX	20/71	28	39
5-FU	13/44	30	36
MTX	9/50	18	35
ADR	9/66	14	34
cis-DDP	3/43	7	23

*Criterion for activity: average $\Delta TS < -1.0$ omu between Days 0 and 6.

activity of this drug as evaluated by the assay compares favorably with CTX, one of the standard agents tested concurrently against 71 of the same 80 tumors. The response rate of CTX was 28%, with an average tumor regression of 39%. The four additional standard agents most commonly tested, although assayed against a smaller number of tumors, were less active than TCNP. The antimetabolite 5-FU showed activity similar to that of CTX, while MTX and ADR showed activity similar to that of HHT. The least activity was shown by *cis*-DDP.

Thirty-two of the 80 tumors (40%) assayed were unresponsive to all drugs tested. Nine percent of the tumors (seven of 80) were responsive only to HHT, TCNP, or both. The singularity in response of essentially unresponsive tumors to new agents attests to the sensitivity of the SRC assay as an in vivo method for detecting activity of new chemotherapeutic agents.

A large proportion (71%) of the total tumors assayed included the solid cancers, ie, breast, lung, ovarian, colorectal, and cervical. Table 7 lists percent response rates by these histologic types, with the phase I drugs and standard agents tested against each type. Differences in response among these five tumor types to HHT and TCNP were evident. With TCNP, response rates were 21% (breast), 42% (lung), 70% (ovarian), 38% (colorectal), and 88% (cervical). With regard to the less active agent HHT, response rates were 5% (breast), 25% (lung), 30% (ovarian), 0% (colorectal), and 25% (cervical). TCNP was the most active single agent tested against cervical and ovarian tumors. It was less active against breast, lung, and colon tumors, with activity similar to the standard most active agent tested. It would appear that some histologic types are more sensitive to TCNP than others.

DISCUSSION

First transplant generation xenografts (fresh surgical explants) of human tumors were used in the 6-day SRC assay to screen two phase I drugs, HHT and TCNP, for activity against a broad spectrum of histologic tumor types. The compounds exhibited a threefold difference in activity when tested against the same panel of 80 human tumors, producing response rates of 16% for HHT and 45% for TCNP.

Although the assays which constituted the basis of this report were carried out with the objective of cor-

TABLE 7.—Comparison of the response of human tumors to TCNP and HHT and standard agents according to histologic type*

Drug	% response by histologic type (No. of responsive tumors/No. tested)				
	Breast	Lung	Ovarian	Colorectal	Cervical
TCNP	21 (4/19)	42 (5/12)	70 (7/10)	38 (3/8)	88 (7/8)
HHT	5 (1/19)	25 (3/12)	30 (3/10)	0 (0/8)	25 (2/8)
CTX	26 (5/19)	25 (3/12)	56 (5/9)	29 (2/7)	50 (4/8)
5-FU	16 (3/19)	—	40 (4/10)	17 (1/6)	—
MTX	6 (1/18)	33 (4/12)	—	—	38 (3/8)
ADR	11 (2/19)	17 (2/12)	10 (1/10)	—	25 (2/8)
cis-DDP	—	17 (2/12)	10 (1/10)	—	0 (0/7)
L-PAM	5 (1/19)	—	—	—	—
BLEO	—	10 (1/10)	—	—	—
VCR	—	—	—	17 (1/6)	43 (3/7)
Mit-C	—	—	—	0 (0/6)	—
SZC	—	—	—	0 (0/6)	—
TT	—	—	56 (5/9)	—	—

*Criterion for activity: average $\Delta TS < -1.0$ omu between Days 0 and 6.

relating assay results with clinical responses in retrospective and prospective clinical studies, it was possible to insert the phase I drugs for testing in parallel with standard chemotherapeutic agents in the same assay. With the inclusion of phase I and clinically active agents in the same assays, comparisons of activity could be made using clinically active agents as a baseline. The clinically relevant parameters, response rate and degree of tumor reduction, were applied in a comparison of the activity of HHT and TCNP with CTX. CTX was selected as the primary standard clinical agent for this comparison, since it had been tested against the largest proportion (89%) of the tumors assayed. The order of ranking of relative activity for the phase I drugs and CTX was TCNP > CTX > HHT, with response rates of 45%, 28%, and 16%, respectively. The average tumor regression values for responsive tumors were 45% (TCNP), 39% (CTX), and 38% (HHT). The high response rate and percent tumor regression indicated TCNP to be superior to HHT in overall activity and slightly more active than CTX.

Although the relative activity of TCNP was greater than that of standard agents when a spectrum of tumor types were considered, differences in sensitivity to TCNP were evident among the five major histologic tumor types, suggesting organ type specificity.

A factor critical in the design of the SRC assay as an in vivo method for evaluating drug activity is the use of tumor fragments rather than cell suspensions. In contrast to dispersed cell populations, the tumor fragment retains cell membrane integrity, cell-to-cell contact, and the spatial relationship of the heterogeneous cell populations, providing intact permeability barriers necessary for new drug testing and permitting determination of the net response to drug treatment by direct measurement. The tumor fragment/body weight ratio also more closely mimics a residual tumor following reductive surgery or a micrometastasis, states of clinical disease which would ordinarily be managed by chemotherapy.

In the SRC assay, each xenograft serves as its own baseline for evaluating drug activity. Precise measurement under magnification of an initial and final size and calculation of a ΔTS value permit direct determination of the drug effect on the tumor graft. As in the clinic, for a drug to be considered active it must induce a measurable degree of regression in the assay. The average control group ΔTS value is not utilized in evaluating antitumor effects but serves as a monitor of tissue quality and growth potential. Assays with unsatisfactory growth can thus be designated as being of questionable validity.

The high evaluable assay rate of 90% obtainable with the SRC methodology is of utmost importance in that it permits accumulation of substantial quantities of valid test data required to conduct comparative drug evaluation studies of the nature presented in this report.

As demonstrated in this study, it is feasible to evaluate the activity of phase I drugs relative to clinically active agents using fresh surgical explants of human tumors in a 6-day assay period. Application of the SRC assay in this manner, while new chemotherapeutic agents are still undergoing phase I clinical toxicology trials, could permit establishment of priorities for entry of phase I agents into phase II clinical trials on the basis of superior activity as shown by assay response rates or spectrum of antitumor activity. However, validation of the SRC assay to predict potential clinical activity of new chemotherapeutic agents will ultimately depend on the results of phase II trials for the two drugs tested.

REFERENCES

1. Bogden AE, Kelton DE, Cobb WR, et al. A rapid screening method for testing chemotherapeutic agents against human tumor xenografts. *In* Proceedings of the Symposium on the Use of Athymic (Nude) Mice in Cancer Research (Houchens DP, and Ovejera AA, eds). New York, Gustav Fischer, Inc, 1978, pp 231–250.
2. Bogden AE, Houchens DP, Ovejera AA, et al. Advances in chemotherapy studies with the nude mouse. *In* The Nude Mouse in Experimental and Clinical Research (Fogh J, and Giovanella BC, eds). New York, Academic Press, 1982, vol 2, pp 367–400.
3. Bogden AE, Cobb WR, and LePage DJ. An in vivo method for testing chemotherapeutic agents against first transplant generation human tumor xenografts. *In* Design of Models for Testing Cancer Therapeutic Agents, Litton Bionetics Workshop Series No. 3 (Fidler IJ, and White RJ, eds). New York, Van Nostrand Reinhold Co, 1982, pp 175–184.
4. Bogden AE, Cobb WR, LePage DJ, et al. Chemotherapy responsiveness of human tumors as first transplant generation xenografts in the normal mouse: six-day subrenal capsule assay. Cancer 48:10–20, 1981.
5. Powell RG, Weisleder D, and Smith CR, Jr. Anti-tumor alkaloids from *Cephalotaxus harringtonia:* structure and activity. J Pharm Sci 61:1227–1230, 1972.
6. Townsend LB, Lewis AF, and Roti Roti LW. Synthesis of 6-amino-4-methyl-8-(beta-D-ribofuranosyl)pyrrolo(4,3,2-de)pyrimido (4,5-c) pyridazine-5'-phosphate and its utility against L-1210 mouse leukemia. Chem Abstr 89:44130j, 1978.

Action of (*S*)-10-Hydroxycamptothecin on P388 Leukemia and Distribution of the Drug in Mice[1,2]

W. A. Creasey,* M. Richards, D. Gil, and K. C. Tsou[3]

As an inhibitor of the growth of P388 leukemia in mice, (*S*)-10-hydroxycamptothecin (OPT) was as potent as the parent compound camptothecin (CPT). Incorporation of thymidine into DNA was the parameter most sensitive to OPT in vitro (ED50 ~ 4 μg/ml), but incorporation of cytidine into RNA and of acetate into lipids was also reduced significantly in the presence of the drug. The cytofluorometric profile suggested suppression of the S and G_2/M phases. The distribution of OPT in mice at 2 and 24 hours after ip injection (10 mg/kg) was essentially similar to that of CPT, with the exception of a somewhat greater concentration of CPT in the liver. In their pharmacology, OPT and CPT appear to be very similar, despite reports that the hydroxy derivative is less toxic. [Cancer Treat Rep 67:179–182, 1983]

(*S*)-10-Hydroxycamptothecin (OPT) is a naturally occurring congener of camptothecin (CPT) that is found in the tree *Camptotheca acuminata* (1,2). Although CPT was very promising in preclinical trials (3,4), in the clinic its severe toxicity, especially to the liver and gastrointestinal tract, has precluded further use (5–7) except possibly for nonsystemic application to treat bladder cancer (8). Initial study of OPT, on the other hand, suggests that it might be less toxic than the parent drug (9,10), and thus further exploration of this derivative is justified. This report details experiments carried out using P388 leukemia; some of the data have appeared in abstract form (11).

MATERIALS AND METHODS

OPT was provided as the Na salt by the Department of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences. P388 leukemia was maintained in $CD2F_1$ mice by weekly ip inoculation of 10^6 cells/animal. For measurements of sensitivity to drug in vivo, ip treatments were started 1 day after transplantation and were continued for 5 days; the animals were weighed daily. Cells required for biochemical studies were harvested 5 days after transplantation, erythrocytes were lysed by exposure to hypotonic saline, and the cell pellet was resuspended in Eagle's minimal essential medium supplemented with 12% dialyzed calf serum; the same medium was used for cell incubations.

The procedures used to study the incorporation of labeled precursors ([methyl-^{3}H]thymidine, 3 Ci/mmol; [^{3}H]cytidine, 27.6 Ci/mmol; [^{14}C]acetate, 50 mCi/mmol; [^{14}C]phenylalanine, 424 mCi/mmol; and [^{14}C]glucose, 7.8 mCi/mmol) have been described in earlier publications (12–14), with the exception of the substitution of Eagle's medium for buffered saline. A 15-minute preincubation of the cells with drug was followed by incubation for 30 and 60 minutes with labeled precursor. Except for [^{14}C]acetate incorporation into lipids, the reaction was terminated, and soluble radioactivity was removed by washing with cold 5% (wt/vol) trichloroacetic acid. Release of $^{14}CO_2$ from [^{14}C]glucose was studied in Warburg flasks with the tracer in a final volume of 2 ml. The $^{14}CO_2$ was trapped on filter paper moistened with 0.1 ml of 1 M NaOH in the center well. Lipids labeled with [^{14}C]acetate were extracted with ethanol-ether (3:1, vol/vol), and after evaporation, the dried extract was taken up in petroleum ether.

Gel filtration studies and experiments using equilib-

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rium dialysis were employed to assess the interaction between calf thymus DNA (Sigma Type I) and drug. These procedures have been described previously (13,14), except that dialysis cells were used; corrections were applied for dilution and binding to the membrane.

Cytofluorometric analysis, using the DNA-specific fluorescent diamidino dye, 4,6-diamidine-2-phenylindole, was carried out as described elsewhere (10). An ICP-22 flow cytophotometer (Ortho Instruments, Westwood, MA) was equipped with a high-pressure 100 W mercury arc lamp, a KG-1 heat filter, a BG-38 infrared blocking filter, a UG-1 (360-nm peak transmission) excitation, and fluorescein isothiocyanate special barrier (450–490-nm bandpass) filter for emission.

For studies on the tissue distribution of OPT, mice received ip injections of 10 mg/kg and were killed by cervical dislocation 2 and 24 hours later. Tissues were dissected out and homogenized with 2 vol of water, and the homogenates mixed with equal volumes of 3 N HCl. The total acidified samples were then shaken with 3 vol of water-saturated ethyl acetate. After centrifuging, the organic layer was examined for fluorescence using either an Aminco-Bowman spectrophotofluorometer (378-nm excitation, 425-nm emission) or a Turner fluorometer (filters 7-60 for excitation and 47B for emission). This is essentially the method used by Creaven et al (15) for CPT, but toluene used by those investigators did not extract OPT efficiently, and thus ethyl acetate, which extracted an average of 93% of the drug, was substituted. Thin-layer chromatography of the extracts on 0.5-mm layers of silica gel H was carried out in ascending fashion with chloroform-ethanol (15:1, vol/vol) and ethyl acetate-isopropanol (1:1, vol/vol) as described previously in studies with CPT (16). Chromatograms were scanned with a Camag attachment to the Turner fluorometer.

RESULTS AND DISCUSSION

At daily doses of 2 mg/kg of OPT the increase in lifespan of mice bearing P388 leukemia was 128%; this compared with 135% for similar animals treated with CPT. Weight changes (maximum losses of 5% and 8% of body weight) did not differ between the groups. Thus, the two drugs are rather comparable in their potency. Shortage of OPT precluded a complete evaluation of their dose-response curves and toxic effects.

Cytofluorometric analysis was carried out on cells exposed to OPT (10 μg/ml) for 3 hours in vitro or harvested from mice after ip injections of 2 mg/kg. The

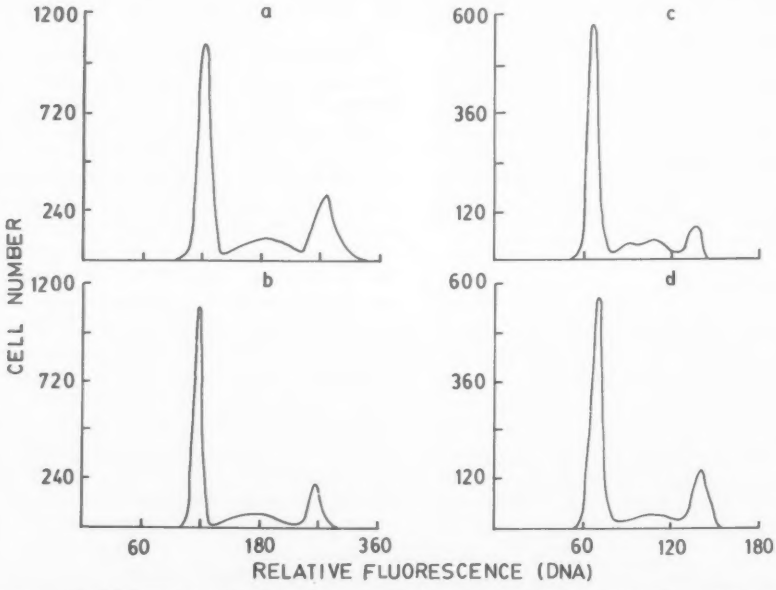

FIGURE 1.—Cytofluorometric profile of nuclei derived from control and OPT-treated P388 cells. a. Control: G_1, 42.64%; S, 42.20%; G_2, 15.15%. b. 3 hrs in vitro with OPT at 10 μg/ml: G_1, 41.11%; S, 38.87%; G_2, 20.01%. c. 3 hrs after 2 mg/kg in vivo: G_1, 49.62%; S, 39.16%; G_2, 11.21%. d. 24 hrs after 2 mg/kg in vivo: G_1, 45.90%; S, 29.85%; G_2, 24.24%. The data are suggestive of suppression of S phase with a later developing block at G_2/M.

TABLE 1.—Effect of OPT on biosynthetic pathways in P388 cells in vitro*

OPT (μg/ml)	Thymidine incorporation into DNA	Cytidine incorporation into DNA	Phenylalanine incorporation into protein	Acetate incorporation into lipid	Glucose oxidation to CO_2
0.5	96.6	89.2	106.4	99.2	102.1
1.0	65.9	82.2	102.5	77.7	101.4
10.0	37.6	51.7	106.6	73.1	93.1
50.0	13.2	42.0	100.8	69.9	70.2

*Data are the mean of 3 experiments performed in duplicate, with values for both 30- and 60-min incubation times included.

data for both types of exposure were consistent with suppression of the S and G_2/M phases as has been described for OPT in hepatoma cells (10) and for CPT in human lymphocytes stimulated by phytohemagglutinin (4). Typical plots appear in figure 1. The data were corrected using an Apple computer and a program developed by W. Tsou based on the equations of Wood and Todd (17).

Table 1 shows the inhibition of biochemical pathways in P388 cells in vitro when they were incubated with OPT. Incorporation of [^{14}C]phenylalanine into residual protein and the oxidation of [^{14}C]glucose to CO_2 were largely unaffected by drug at concentrations up to 50 μg/ml. DNA synthesis was the pathway most sensitive to OPT, but the incorporation of [^{3}H]cytidine into RNA was also significantly inhibited. It should be stressed that examination of the acid-soluble washings from cells that had been separated from medium by centrifugation through isotonic sucrose solution in Shevky-Stafford and McNaught tubes indicated that uptake of labeled precursors by the cells was unaffected by OPT. When cells preincubated with OPT (50 μg/ml) were spun down, washed twice with medium, and then resuspended and incubated with [^{3}H]thymidine, incorporation of precursor into DNA was restored to about 90% of control values. This ready reversibility of the inhibition, as well as the selective effect on nucleic acid biosynthesis, is very similar to the pattern described for CPT (18).

Binding of OPT by calf thymus DNA could be demonstrated readily by both gel filtration and equilibrium dialysis. Using the latter technique, Scatchard plots (fig 2) suggested that while the affinity of OPT for this DNA

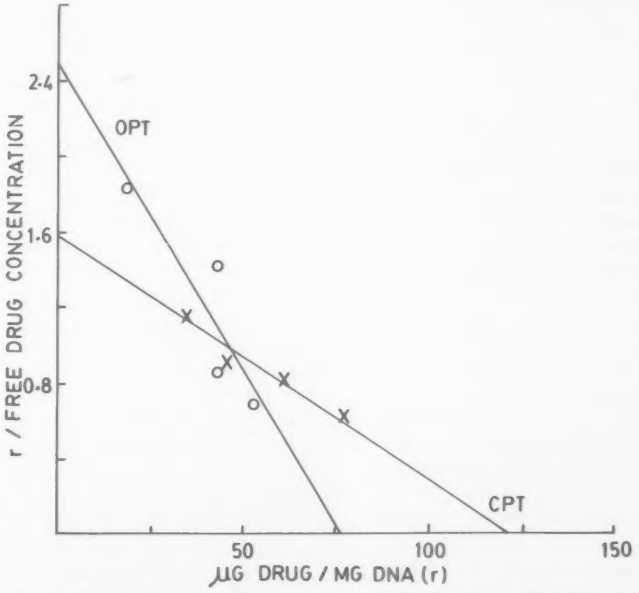

FIGURE 2.—Scatchard plots of equilibrium dialysis data for the binding of OPT by calf thymus DNA. Equilibrium was achieved by dialysis for 12 hrs at room temperature in the dark, using cells with 5-ml capacity on each side of the membrane. The DNA concentration was 0.4 mg/ml.

TABLE 2.—Distribution of OPT in mice*

Tissue	Concentration (μg/g or μg/ml)	
	2 hrs	24 hrs
Brain	0	0.03†
Bile	8.00 ± 2.61	3.05 ± 1.65
Blood	0.86 ± 0.30	0.07 ± 0.03
Fat	0.12 ± 0.07	0.23 ± 0.10
Feces	6.4 ± 3.50	11.62 ± 4.90
Intestine	1.13 ± 0.27	0.81 ± 0.47
Kidney	0.18 ± 0.08	0.03†
Liver	0.58 ± 0.18	0.07 ± 0.05
Urine	179.57 ± 47.36	19.30 ± 6.20

*Data are the mean ± SD of triplicate determinations on 3 mice. Animals received 10 mg/kg of OPT.

†Only 1 value available.

preparation was greater than that of CPT at neutral pH (dissociation constants, 8.8×10^{-5} M vs 2.2×10^{-4} M), the binding capacity of the DNA was greater for CPT than for OPT (0.36 vs 0.22 μmol/mg).

Tissue distribution of OPT was studied in mice injected ip with 10 mg/kg of the drug; the data appear in table 2. It is evident that urinary excretion occurs rapidly during the first 2 hours with up to 10% of the dose cleared by that route; this value rose to about 35% by 24 hours. There was a steady elimination in the stools. Chromatography of urinary extracts showed that at 2 hours, 95% of the fluorescent material was present as unchanged drug; at 24 hours, 83% was OPT. Incubation of urine for up to 24 hours with β-glucuronidase (Sigma Type B-1) prior to extraction did not increase the amount of fluorescent material extracted from the 2-hour urine sample and led to only a 5% increase in the 24-hour sample. Thus, despite the presence of the 10-hydroxyl substituent, formation of glucuronides does not seem to be a major pathway for the metabolism of OPT in mice. In comparing our data with similar studies of CPT (19), it is evident that the pharmacokinetic disposition of the two drugs is very similar, with the possible exception of what may be less retention of OPT at 24 hours in our experiments. We compared tissue drug concentrations of CPT in mice receiving the same dose as that used for OPT. The range of values obtained did not differ from those for OPT, with the exception that in the liver higher levels of CPT were recorded (0.10–0.45 μg/g); the significance of this difference from the point of view of the reported lower toxicity of OPT cannot be assessed at present.

Our studies indicate that the pharmacology of OPT closely resembles that of its congener CPT in mice. With the possible exception that there might be less hepatic retention of OPT, the data provide no clear-cut basis for the biologic findings that the drug is less toxic than CPT. Further work on this specific aspect is indicated.

REFERENCES

1. WANI MC, and WALL ME. Plant antitumor agents. II. The structure of two new alkaloids from *Camptotheca acuminata*. J Org Chem 34:1364–1367, 1969.
2. HSU JS, CHAO TY, LIN LT, ET AL. Chemical constituents of the anticancer plant *Camptotheca acuminata* Decne II. Chemical constituents of the fruits of *Camptotheca acuminata* Decne. Acta Chim Sinica 35:193–200, 1977.
3. WALL ME, WANI MC, COOK CE, ET AL. Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. J Am Chem Soc 88:3888–3890, 1966.
4. GALLO RC, WHANG-PENG J, and ADAMSON RH. Studies on the antitumor activity, mechanism of action and cell cycle effects of camptothecin. JNCI 46:789–795, 1971.
5. GOTTLIEB JA, GUARINO AM, CALL JB, ET AL. Preliminary pharmacologic and clinical evaluation of camptothecin sodium (NSC-100880). Cancer Chemother Rep 54:461–470, 1970.
6. MUGGIA FM, CREAVEN PJ, HANSEN HH, ET AL. Phase I clinical trial of weekly and daily treatment with camptothecin (NSC-100880): correlation with preclinical studies. Cancer Chemother Rep 56:515–521, 1972.
7. MOERTEL CG, SCHUTT AJ, REITEMEIER RJ, ET AL. Phase II study of camptothecin (NSC-100880) in the treatment of advanced gastrointestinal cancer. Cancer Chemother Rep 56:95–101, 1972.
8. TUNG H, CHING-CHIANG Y, HSIEN-YOU C, ET AL. Camptothecin instillation and local injection for prevention and treatment of urinary bladder tumors. Chin Med J 92:57–60, 1979.
9. XU (HSU) B, CHEN JT, YANG JL, ET AL. New results in pharmacologic research on some anticancer agents. US-China Pharmacology Symposium, Washington, DC, Oct 29–31, 1979.
10. TSOU KC, XU (HSU) B, MILLER EE, ET AL. Antitumor effect of (*S*)-10-hydroxycamptothecin on mouse hepatoma BW 7756 and its possible mode of action. Anticancer Res 1:115–119, 1981.
11. CREASEY WA, RICHARDS M, GIL D, ET AL. Pharmacologic and cytokinetic studies with (*S*)-10-hydroxycamptothecin. Proc Am Assoc Cancer Res and ASCO 22:235, 1981.
12. CREASEY WA. Biochemical effects of the vinca alkaloids, IV. Studies with vinleurosine. Biochem Pharmacol 18:227–232, 1969.
13. ———. Biochemical effects of *d*-tetrandrine and thalicarpine. Biochem Pharmacol 25:1887–1891, 1976.
14. ———. Biochemical effects of berberine. Biochem Pharmacol 28:1081–1084, 1979.
15. CREAVEN PJ, ALLEN LM, and MUGGIA FM. Plasma camptothecin (NSC-100880) levels during a 5-day course of treatment: relation to dose and toxicity. Cancer Chemother Rep 56:573–578, 1972.
16. HART LG, CALL JB, and OLIVERIO VT. A fluorometric method for determination of camptothecin in plasma and urine. Cancer Chemother Rep 53:211–214, 1969.
17. WOOD JCS, and TODD P. Analysis of cellular DNA distributions, I. G_2/G_1 peak channel ratios. Cell Biophys 1:211–218, 1979.
18. HORWITZ SB. Camptothecin. *In* Antineoplastic and Immunosuppressive Agents II (Sartorelli AC, and Johns DG, eds). Berlin-Heidelberg-New York, Springer-Verlag, 1975, pp 649–656.
19. GUARINO AM, ANDERSON JB, STARKWETHER DK, ET AL. Pharmacologic studies of camptothecin (NSC-100880): distribution, plasma protein binding, and biliary excretion. Cancer Chemother Rep 57:125–140, 1973.

Maytansine as a Cause of Acute Proliferative Disorder of Bladder Urothelium in the Rat[1]

Ralph deVere White,* Michael O'Brien, John Krikorian, Richard Babayan, and Carl A. Olsson[2]

Male Wistar Lewis rats were injected with maytansine at a total dose of 0.425 to 0.6 mg/kg over a 3-week period. Biopsies taken at 5 weeks showed that 23% of the bladders had hyperplasia or other abnormalities. Of the bladder biopsies done for the first time at 8 weeks, 80% demonstrated papillary hyperplasia. A small number of biopsies at 13 and 19 weeks still showed hyperplasia and minimal inflammatory reaction. These results were seen regardless of prior biopsy, the presence of bladder calculi, and/or the dose level used. It is not clear from the data whether the observed changes are reversible or precarcinogenic. However, these changes closely resembled the histologic features described in the evolution of bladder cancer in rats fed *N*-C4-(5-nitro-2-furyl)-2-thiazolyl formamide. [Cancer Treat Rep 67:183–184, 1983]

In testing the effectiveness of maytansine against a spontaneously arising renal adenocarcinoma in a male Wistar Lewis rat, we encountered alterations in the bladder urothelium not previously described. Since these alterations resembled those reported during bladder carcinogenesis, we investigated the effect of maytansine on the bladder urothelium of non-tumor-bearing rats.

MATERIALS AND METHODS

Male Wistar Lewis rats (Charles River) were given Camm rat chow and water ad libitum. Maytansine was administered ip, and rats were killed at intervals following the first injection.

Group 1.—Ten animals bearing flank renal tumors received two ip injections of maytansine (0.25 mg/kg, Week 0; 0.175 mg/kg, Week 1). While the tumors were being removed (5 weeks after the first injection) it was noted that one-half of the animals had urinary retention. One bladder was submitted for histologic examination.

Group 2.—Ten additional, tumor-free, Wistar Lewis rats received three ip injections of maytansine (0.25 mg/kg, Week 0; 0.175 mg/kg, Weeks 2 and 4). Five bladder biopsies were performed at Week 5 (bladder closed with 6.0 Dexon), and three additional biopsies were done at Week 8.

Group 3.—Ten additional tumor-free rats received two ip injections of maytansine (0.25 mg/kg, Week 0; 0.175 mg/kg, Week 2). Seven bladder biopsies were performed at Week 5. Six animals remained alive at 8 weeks and another biopsy was done in these bladders. At 13 and 19 weeks, additional bladder biopsies were performed in the two animals that were alive (bladder closed with 6.0 Dexon).

Group 4.—Ten additional tumor-free rats received three ip injections of maytansine (0.25 mg/kg, Week 0; 0.175 mg/kg, Weeks 2 and 4), and another ten received 0.5 ml of saline ip at Weeks 0, 2, and 4. The 20 animals were killed at Week 8 following the initial injections.

In each case, tissue obtained for light microscopy was fixed in 10% Formalin, embedded in paraffin, and stained with hematoxylin and eosin for reading by a pathologist (M. O'Brien).

RESULTS

Data from Groups 1–3 are combined. Separate results are reported for Group 4. In Groups 1–3, at 5 weeks, bladder biopsies were performed in 13 animals; one bladder contained calculi. The urothelium in ten bladders, all from non-tumor-bearing rats, was normal. One

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of the remaining three bladders (from an animal that had received a total of 0.425 mg of maytansine) demonstrated extensive necrosis of the epithelium, which had been replaced by a fibrinopurulent exudate. The remaining two bladders showed ulceration and re-epithelialization of the ulcer surface. In both bladders, a significant residual inflammation was still present, represented by edema, vascular dilation, and polymorphonuclear infiltration of the submucosa. The regenerated epithelium was hyperplastic (four to eight cells in thickness). The cells in the re-epithelialized mucosa showed nuclear enlargement, with coarse chromatin, prominent nucleoli, and frequent mitotic figures. Both animals had received 0.425 mg/kg of maytansine.

In nine bladders, repeat biopsies were done 8 weeks after the initial injection of maytansine. One biopsy showed mucosal necrosis; the remaining eight demonstrated papillary hyperplasia. Five bladders contained stones. Three animals had received 0.425 mg/kg and five had received 0.6 mg/kg of maytansine. The papillary hyperplasia was similar in the bladders with or without calculi; it was not influenced by the two different maytansine doses. The papillary cores contained delicate capillaries, many of which were directly apposed to the overlying epithelium. The epithelial hyperplasia was more prominent than that existing at 5 weeks, even though inflammatory reaction in the submucosa had largely subsided. At both 13 and 19 weeks following the initial maytansine injection (0.425 mg/kg), two animals were alive. One bladder contained stones. Biopsies were performed in both bladders. At 13 weeks both biopsies showed urothelial papillary hyperplasia. At 19 weeks, both biopsies showed a very modest inflammatory infiltrate consisting of scattered monocytic cells in the mucosa and submucosa with an occasional lymphoid nodule within the basal portion of the epithelium. The epithelium remained eight to ten cells in thickness, with a slight tendency toward surface maturation. Atypical cytologic characteristics such as enlarged nuclei and prominent nucleoli were widespread. The nuclear chromatin pattern was less coarse than that seen in biopsies taken at earlier periods, and mitotic figures were less frequently evident.

In Group 4, the saline control bladders were all normal, by light histology, while eight of the ten maytansine-treated bladders showed hyperplastic changes similar to those observed in the earlier experiments.

DISCUSSION

This report is a preliminary communication, suggesting the potential for urothelial abnormalities induced by the cytotoxic agent maytansine. Of the bladder biopsies done at 5 weeks, 23% showed significant urothelial abnormalities; 80% of the bladder biopsies performed for the first time at 8 weeks demonstrated pathologic changes. Bladders from some of the animals that had repeat bladder biopsy and closure with absorbable suture material contained calculi. However, urothelial papillary hyperplasia was evident in the bladders of animals that had no calculi and had had no previous biopsies.

Many of the histologic features noted following maytansine administration are similar to the urothelial proliferation known to occur following the administration of a single dose of cyclophosphamide (1). On the other hand, the atypical hyperplasia following maytansine persisted even in long-term (19 weeks) studies. This persistence of urothelial hyperplasia is in contrast to the study of Locher and Cooper (1), in which bladder epithelium returned to normal thickness in most areas by 10 weeks following cyclophosphamide.

The histologic changes following maytansine also share features described by Tiltman and Friedell (2) in their discussion of the evolution of bladder cancer in male Fischer rats fed *N*-C4-(5-nitro-2-furyl)-2-thiazolyl formamide (FANFT). As noted by these authors, outfoldings of hyperplastic epithelium with the formation of micropapillae were evident by 8 weeks. In their studies, urothelial papillary hyperplasia preceded the development of malignant transformation, and transitional cell cancer invasive to the bladder muscle layers was noted by 22 weeks. In the FANFT study, animals were continually fed carcinogen over the course of the experiment.

The study demonstrated prominent hyperplasia after only two injections of maytansine. This study does not resolve whether the papillary hyperplasia represents a reversible process or a premalignant condition. Fukushima et al (3) have shown that similar histologic response follows surgical incision of the bladder and subsequent suturings. However, these bladders returned to normal in 4 weeks. In the animals that had repeat biopsy, the surgical intervention may, therefore, have played a role in the histologic findings. However, the abnormal papillary hyperplasia was present in bladders that had not had previous biopsy.

REFERENCES

1. Locher GW, and Cooper EH. Repair of rat urinary bladder epithelium following injury of cyclophosphamide. Invest Urol 8:116–127, 1970.
2. Tiltman AJ, and Friedell GH. The histogenesis of experimental bladder cancer. Invest Urol 9:218–226, 1971.
3. Fukushima S, Cohen SM, Arai M, et al. Scanning electron microscopic examination of reversible hyperplasia of the rat urinary bladder. Am J Pathol 102:373–380, 1981.

Repeated Femoral Vein Cannulation for Administration of Chemotherapeutic Agents[1,2]

Bernard D. Nidus,[3,*] James L. Speyer,[4] Joseph Bottino,[4] Michael Green,[4] Mark Levin,[4] and Franco M. Muggia[4,5]

A cannulation set has been designed for repeated short-term infusion of vesicant chemotherapeutic agents via the femoral vein. The major complication was thrombophlebitis in 2.1% of infusions. The procedure provides reliable venous access when therapeutic plans are changed or when the inability to provide catheter care makes an indwelling catheter unwarranted. [Cancer Treat Rep 67:185–186, 1983]

Vesicant chemotherapeutic drugs can produce sclerosis of peripheral veins and, if extravasated, severe tissue necrosis. The drugs should, therefore, be infused via a secure route, preferably a large vein with rapid blood flow. This report presents our experience with infusions of these agents by repeated femoral vein cannulation (RFVC) utilizing the Seldinger technique (1).

METHODS

The cannulation set consists of a 19-gauge needle; wire guide; and 5 Fr, 15-cm catheter (CTS-500, Cook, Inc, Bloomington, IN). For very obese patients a wider bore set is used: 18-gauge needle and 6.3 Fr catheter (CTS-600).

The patient is recumbent and the leg is abducted; aseptic technique and local anesthesia are used. The site for puncture is at the groin crease, one finger's breadth medial to the femoral pulse. A skin incision is not required. The needle is introduced quickly to its full length, bevel up and cephalad, at an angle of 45°–80° to the thigh. It is then slowly withdrawn while aspirating with a 12-ml syringe. Alternatively, the needle may be advanced slowly while aspirating. When venous blood is freely drawn, the soft-tipped wire guide is passed through the needle and into the vein. If the wire does not slide easily into the vein, the angle between the needle and thigh is reduced or the puncture is repeated. The wire is then advanced without force for a distance of about 20 cm and the needle removed. The femoral catheter is held near its tip and threaded over the wire, through the skin and into the vessel. To avoid losing the wire within the vein, a segment of wire not covered by the catheter is kept visible throughout the procedure. The wire is removed, and the position of the catheter is confirmed by aspirating venous blood. Catheters to be retained for > 6 hours are sutured to the skin and the puncture site is treated with povidone and dressed daily.

The catheter is suitable for infusion, transfusion, and blood drawing. An infusion pump is employed to administer chemotherapeutic agents. Patients remain in bed when receiving drug infusions but are allowed to sit in a chair and take short walks when receiving 5% dextrose or normal saline to maintain catheter patency. After the catheter is removed finger pressure at the puncture site is maintained for 2 minutes and a 4.5-kg sand bag is applied for 20 minutes; the patient is then permitted to resume normal activity.

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TABLE 1.—Clinical experience with femoral catheters

Drug schedule	No. of — Patients	Infusions	Complications*
Doxorubicin, 6 hrs	48	134	2 with phlebitis, 1 with sepsis
Doxorubicin, 24 hrs	26	85	2 with phlebitis
Bisantrene, daily × 5	13	21	1 with phlebitis
Vindesine, daily × 2	10	14	2 with phlebitis
4'-Epi-doxorubicin, 6 hrs	23	174	2 with phlebitis
Totals	120	428	

*Values = No. of patients.

RESULTS

After preparation, cannulation took from 1 to 5 minutes; one to two punctures usually sufficed. Cannulations were generally alternated from side to side except where surgical scars, fistula, tumor, adjacent ostomy, or previous phlebitis dictated otherwise. In the rare instance when the femoral artery was punctured inadvertently, pressure was applied for several minutes. Under the supervision of one of the authors (B. D. Nidus), eight individuals became proficient in the procedure. One hundred and twenty patients were treated with a total of 428 cannulations (range, one to 11 per patient; table 1). Three very obese patients required the larger bore set. *All* patients were successfully cannulated.

There were no instances of extravasation, hemorrhage, or hematoma, and no catheter was dislodged. The major complication was ileofemoral thrombophlebitis without pulmonary embolism, occurring from 1 to 4 days after the infusion was terminated. It was diagnosed clinically in nine patients and confirmed by venogram in seven. One other patient developed fever and staphyloccocal septicemia 5 hours after catheter introduction.

DISCUSSION

The low incidence of sepsis related to the femoral cannula in our patients can be attributed to aseptic technique, daily povidone treatment, and short duration of cannulation. Extravasation of solution was precluded by inserting the catheter to its full length within the vein and confirming its position by aspirating venous blood. In a recent report the complication rate of RFVC was 0.1% in 12 dialysis patients, each undergoing an average of 200 cannulations (2). The relative safety of the procedure in patients with defective coagulation is attested to by the lack of untoward bleeding in scores of fully heparinized dialysis patients receiving RFVC (Nidus BD, unpublished data).

Ileofemoral thrombophlebitis proved to be the one serious complication occurring in 2.1% of the infusions and in 7.5% of patients. Though the numbers were small, there is the suggestion that the incidence of phlebitis was increased when the pelvis had been irradiated, when there was bulk pelvic tumor, or when very sclerotic drugs [bisantrene (3); vindesine (4)] were infused.

In patients with neoplasm, the Hickman catheter, which terminates in the right atrium, can provide long-term vascular access with minimal risk of phlebitis. However, this device necessitates construction of a subcutaneous tunnel and requires patient reliability for a regimen of daily skin care and heparin infusion. Additionally, the catheter can serve as a portal for infection, particularly during episodes of neutropenia (5,6). The femoral catheter in contrast is a short-term device that does not require the patient's participation and is usually removed prior to the occurrence of drug-induced neutropenia. The disadvantages of RFVC are requirements for a separate insertion procedure for each course of infusion, reduced patient mobility, and the occasional induction of chemical phlebitis. The latter problem might be alleviated by avoidance of patients at risk (ie, with pelvic disease), use of more dilute infusions, lengthening of the femoral catheter to reach the inferior vena cava which has a higher blood flow, or addition of heparin (drug compatibility must be proven) and steroids to the infusion (7).

REFERENCES

1. SELDINGER SI. Catheter replacement of the needle in percutaneous arteriography—a new technique. Acta Radiol 39:368–379, 1953.
2. NIDUS BD, and NEUSY A. Chronic hemodialysis by repeated femoral vein cannulation. Nephron 29:195–197, 1981.
3. DORR RT, and FRITZ WL. Reference VP 16. *In* Cancer Chemotherapy Handbook (reference on bisantrene included). New York, Elsevier, 1980, p 423.
4. SPIEGEL R, BLUM R, PINTO C, ET AL. Phase I clinical trials of antralenedicarboxaldehyde CL 216942 administered in a 5 day schedule. Cancer Res 42:354–358, 1982.
5. BLACKLOCK HA, HILL RS, CLARKE AG, ET AL. Use of modified subcutaneous right atrial catheter for venous access in leukemic patients. Lancet 1:993–994, 1980.
6. WADE JC, NEWMAN KA, SCHIMPFF SC, ET AL. Two methods of improved venous access in acute leukemia patients. JAMA 246:140–144, 1981.
7. ISAACS JW, MILLIKAN WJ, STACKHOUSE J, ET AL. Parenteral nutrition of adults with a 900 milliosmolar solution via peripheral vein. Am J Clin Nutr 30:552–559, 1977.

Phase II Study of Four-Drug Chemotherapy for Metastatic Neuroblastoma: A Pediatric Oncology Group Study[1,2]

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Cyclophosphamide, vincristine, doxorubicin, and cisplatin, alone or in various combinations of two or three of these agents, have activity against childhood metastatic neuroblastoma (1–4). In an attempt to improve the quality and duration of survival of children with this malignancy, the present study by the Pediatric Oncology Group delineated the toxicity and therapeutic efficacy of the combination of all four of these agents.

MATERIALS AND METHODS

Children between 1 and 21 years of age with stage IV neuroblastoma (5) were eligible if they had had no prior therapy. Normal renal function was required (BUN < 30 mg/dl and creatinine < 1.2 mg/dl for children 5–21 years of age; BUN < 30 mg/dl and creatinine < 1 mg/dl for children 1–4 years of age).

Vincristine (1.5 mg/m^2; maximum dose, 1.5 mg) was given by iv push on Days 1 and 43; cyclophosphamide (500 mg/m^2/dose) was given by 30-minute iv infusion on Days 2, 3, 44, and 45; doxorubicin (30 mg/m^2/dose) was administered on Days 21–23 by iv push; and cisplatin (90 mg/m^2/dose) was given by a 6-hour infusion on Day 63. If a response was observed, this cycle of chemotherapy was repeated every 84 days for six courses or until cumulative doxorubicin had reached 450 mg/m^2. Following this, cycles of chemotherapy (vincristine on Day 1, cyclophosphamide on Days 2 and 3, and cisplatin on Day 22) were repeated every 6 weeks until a total of 2 years of chemotherapy had been given or until the patient had evidence of disease recurrence or progression. Provisions were made for toxicity monitoring and dose modifications and for vigorous hydration associated with the administration of cyclophosphamide and cisplatin (6).

Complete response (CR) was defined as the disappearance of all tumors and radiologic signs of healing of all bony lesions. Partial response (PR) was defined as a decrease in the size of all tumors by $\geq 50\%$, and stability or signs of healing of all bony lesions. A patient was fully evaluable for response if he or she received at least one complete cycle of chemotherapy. Remission duration was defined as zero for the patients who did not have a CR or PR and as the duration of the response in months for those achieving CR or PR. The data for toxicity, response rates, remission duration, and survival were updated as of June 1, 1982.

RESULTS

From November 1979 to October 1981, 43 children (26 males, 17 females: 30 white, 11 black, and two of

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unspecified race) with a median age of 2.75 years (range, 0.95–17.4) were entered in this study. None had received previous therapy. One child received only 3 weeks of chemotherapy in this study (because of parental refusal) and was not included in further analysis. All of the 42 remaining patients were evaluable for toxicity and response.

CRs of 6, 10, 11+, 12, 13, and 15+ months were achieved in six of 42 (14.3%) patients who survived 11+, 21+, 23+, 18+, 18, and 18+ months, respectively, from the start of treatment. Eighteen additional patients (42.9%) achieved a PR. The short remission duration and survival for the total group of 42 patients is reflected by the Kaplan-Meier estimate of 12% CR + PR at 6 months from the start of treatment (SE, 4%) (7). Nineteen of the 42 patients (45%) were alive as of June 1, 1982. The Kaplan-Meier estimates of percent surviving (of all 42 patients) at 6 and 12 months from start of treatment are 71% and 45%, respectively (SE, 7% and 9%).

Forty of the 42 evaluable patients (95%) had at least one toxic effect attributed to at least one chemotherapeutic agent on at least one occasion. The major toxic effects occurring in this study were myelosuppression and gastrointestinal effects. Neutropenia of < 1000 cells/μl occurred in 16 of 42 evaluable patients, with only one of these patients developing an absolute neutrophil count $< 500/\mu$l with one course of therapy. Four patients required admission for parenteral antibiotics to treat infections attributed to chemotherapy-related neutropenia. Thrombocytopenia $< 50{,}000$ cells/μl and anemia with Hgb < 7 g/100 ml occurred with only one course of therapy.

Despite the administration of antiemetics to all patients, nausea and vomiting occurred in 50% (21 of 42 patients). It was observed in relation to cisplatin administration in all 21 patients and less often in association with cyclophosphamide or doxorubicin administration.

Transient renal function abnormalities, including hypomagnesemia, occurred in three patients. All patients received subsequent cisplatin without incident.

The site of the primary tumor, whether or not the patient presented with marrow involvement, the presence or absence of lytic bone lesions at diagnosis, or the presence or absence of increased urinary excretion of catecholamine metabolites were all evaluated as potential prognostic factors. None of them had a statistically significant effect with respect to attainment of response, remission duration, or survival.

DISCUSSION

The therapeutic efficacy of each of these four drugs as a single agent or in previously used combinations (1–4,6,8,9) would not have predicted the poor response rate observed with the sequential administration in this study. The sequence of agents was chosen to allow administration of the maximum tolerated dose of the three myelosuppressive agents (cisplatin, cyclophosphamide, and doxorubicin). Vincristine administration preceded that of cyclophosphamide by 1 day, because that schedule had been shown to have synergistic antitumor effect in an animal tumor model when compared with a simultaneous or reverse sequence of administration of these two agents (10). The results of a recent study, in which a "kinetic" combination of doxorubicin and cisplatin given at less than the maximum tolerated dose for each agent was no more effective than either drug given alone at the maximum tolerated dose (4), confirmed our plans to proceed with the present study in the schedule described. However, our CR plus PR rate of 55% was disappointing when compared with CR plus PR rates of 80% (8) and 71% (9) in other recently reported studies. In the latter study, cyclophosphamide and doxorubicin were given in a kinetic fashion every 3 weeks (7 days of cyclophosphamide orally, followed by doxorubicin on Day 8). A possible explanation for our disappointing results may be the 6–9-week delay between doses of active agents to patients whose tumors, in retrospect, may have been insensitive to one or more of the four agents given. This could allow for regrowth of tumor during intervals between doses of an active agent. In addition, the minimal toxicity of this regimen, given the dismal prognosis of metastatic neuroblastoma, suggests that more closely spaced chemotherapy could be delivered.

REFERENCES

1. Ragab AH, Sutow WW, Komp DM, et al. Adriamycin in the treatment of childhood solid tumors. Cancer 36:1567–1571, 1975.
2. Nitschke R, Starling KA, Vats T, et al. Cis-diamminedi-chloroplatinum (NSC-119875) in childhood malignancies: a Southwest Oncology Group Study. Med Pediatr Oncol 4:127–132, 1978.
3. Sullivan MP, Nora AH, Kulapongs P, et al. Evaluation of vincristine sulfate and cyclophosphamide chemotherapy for metastatic neuroblastoma. Pediatrics 44:685–694, 1969.
4. Nitschke R, Starling K, Lui VKS, et al. Doxorubicin and cisplatin therapy in children with neuroblastoma resistant to conventional therapy: a Southwest Oncology Group study. Cancer Treat Rep 65:1105–1108, 1981.
5. Evans AE, D'Angio GJ, and Randolph J. A proposed staging for children with neuroblastoma. Cancer 27:374–378, 1971.
6. Nitschke R, Fagundo R, Berry DH, et al. Weekly administration of cis-dichlorodiammineplatinum(II) in childhood solid tumors: a Southwest Oncology Group study. Cancer Treat Rep 63:497–499, 1979.
7. Kaplan EL, and Meier P. Non-parametric estimation from incomplete observations. Am Stat Assoc J 53:457–481, 1958.
8. Nitschke R, Cangir A, Crist W, et al. Intensive combined chemotherapy for metastatic neuroblastoma. A Southwest Oncology Group Study. Med Pediatr Oncol 8:281–288, 1980.
9. Green AA, Hayes FA, and Hustu HO. Sequential cyclophosphamide and doxorubicin for induction of complete remission in children with disseminated neuroblastoma. Cancer 48:2310–2317, 1981.
10. Razek A, Vietti T, and Valeriote F. Optimum time sequence for administration of vincristine and cyclophosphamide in vivo. Cancer Res 34:1857–1861, 1974.

Phase II Trial of Spirogermanium for Treatment of Advanced Breast Cancer[1]

Geoffrey Falkson[2,3,*] and Hèndré C. Falkson[3]

In a clinical trial with spirogermanium, responses occurred in seven of 28 women with metastatic breast cancer. All responding patients had had previous exposure to optimal types of chemotherapy. The only side effect was reversible neurotoxicity. [Cancer Treat Rep 67:189–190, 1983]

Spirogermanium, an azaspirane-germanium compound, belongs to a new class of oncolytic agents. It has in vitro cytotoxic activity, and its mode of action appears to be through inhibition of protein synthesis (1). Phase I trials of this agent showed it to be nonmyelotoxic, with responses seen in breast cancer, prostatic cancer, and malignant lymphoma (2–4). The present phase II trial was undertaken to evaluate the effectiveness of spirogermanium in previously treated women with advanced breast cancer.

MATERIALS AND METHODS

Spirogermanium was given to 30 women with histologically documented breast cancer who had measurable and/or evaluable disease. The following clinical measurements and follow-up studies were done prior to treatment: complete history and physical examination, cbc, urinalysis, SMA-12, plasma CEA, chest X-rays and tomograms when indicated, skeletal survey, liver and brain scans if indicated, bone scan, and EEG. These studies were repeated at regular intervals. Response criteria were as follows: *complete remission (CR)*—the disappearance of all evidence of disease; *partial remission (PR)*—a $\geq 50\%$ reduction in size of bidimensionally measurable lesions; *no change*—the failure to qualify for either CR or PR or progressive disease over 8 weeks of therapy; and *progressive disease*—the appearance of new lesions or a $\geq 25\%$ increase of existing lesions. Patient ages ranged from 30 to 73 years (median, 52); Eastern Cooperative Oncology Group performance status was 0–1 in 16 patients and 2–3 in 14; dominant disease sites were bone (14 patients), viscera (11), and soft tissue (five). Estrogen receptor (ER) status was known in seven patients: two were ER-positive, four were ER-negative, and one was ER-equivalent. Spirogermanium was given at a dose of 120 mg/m^2 in a saline infusion over 60 minutes twice weekly. In four patients who received the drug for a prolonged period (> 300 days) the twice weekly schedule was changed to once every 2 or 3 weeks. The median total dose given was 1800 mg and the largest cumulative dose given to an individual patient was 10,570 mg during 563 days.

RESULTS

Toxic effects.—No hematopoietic, gastrointestinal, or renal toxic effects were seen. The only side effects were neurologic: dizziness occurred in 23 patients (in four the dosage had to be reduced because of this). Nonspecific EEG changes were recorded for 17 patients. Other side effects were: drowsiness (six patients), paresthesias (six), ataxia (three), disorientation (two), and nystagmus (one). These side effects were always reversible and lasted only for a few hours. Dizziness, the only dose-limiting toxic effect, occurred during or shortly after the infu-

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TABLE 1.—Characteristics of responding patients*

Patient No.	Performance status	Prior chemotherapy		Dominant lesion	Response to SG	Time to failure (days)
		Agents	Response			
1	1	CMFP	PD	Bone	CR	> 417
2	2	DAVTH/ CMFP	PD	Bone	PR	563
3	1	CMFP DVP	PR PR	Soft tissue	PR	> 513
4	2	CAF	NC	Bone	IMP	318
5	1	CMFVP DAVT	CR PR	Bone	IMP	145
6	1	CAF	PR	Bone	IMP	106
7	2	Oophorectomy + C DAVTH FIVMP	CR NC NC	Bone	IMP	40

*SG = spirogermanium; C = cyclophosphamide; M = methotrexate; F = 5-FU; P = prednisone; D = dibromodulcitol; A = doxorubicin; V = vincristine; T = tamoxifen; H = fluoxymesterone; I = dacarbazine; PD = progressive disease; NC = no change; and IMP = improvement.

sion. It was dose-related and could be diminished by decreasing the rate of the infusion so that dizziness did not last for > 12 hours.

Therapeutic effects.—Twenty-eight patients are evaluable for response. In two patients, spirogermanium was discontinued early because of a local vein reaction (50 mg) in one and a general intolerance to the drug, with flushing and dyspnea (620 mg), in the other. Data for seven responding patients are summarized in table 1. All the responding patients had had prior combination chemotherapy for metastatic disease. Patient 1 had had prior CMFP for ER-negative and progesterone receptor-negative subcutaneous metastases. The subcutaneous metastases did not recur, but the patient showed an unequivocally positive bone scan with widespread lesions, elevated alkaline phosphatase, and pain. During treatment with spirogermanium the results of serial isotope studies gradually improved, and a CR was documented on Day 319 of the trial. In Patient 2, with extensive lytic bone lesions that did not respond to prior combined chemohormonotherapy (including doxorubicin), definite recalcification of lucent lesions occurred with spirogermanium treatment, and a PR was documented by Day 239 of the trial. In four patients with bone metastases (table 1), no change was documented radiologically, but these patients experienced a significant decrease in analgesic requirements accompanied by improvement in performance status. Although these four patients do not meet the full criteria for PR, the delay before radiologic response occurs places the patients in a separate category, best classified as improvement or even PR rather than as no change. Eight patients are classified as having no change while 13 had progressive disease during treatment.

DISCUSSION

Phase I trials with spirogermanium, a unique structural compound, suggest that it has limited and acceptable toxicity, with no myelosuppressive action. Pilot studies conducted in Sweden reported objective responses in various types of malignancy, including breast cancer (1). Results from our own phase II trial confirm that the agent has acceptable neurotoxicity and promising therapeutic efficacy in patients with advanced breast cancer. The major disadvantage of spirogermanium administration appears to be that frequent, slow iv infusions are necessary to achieve adequate blood levels. With 25% of the patients (seven of 28) responding, the 95% confidence interval for response rate is 13%–43%. This response rate in patients with breast cancer resistant to optimal chemotherapeutic regimens, as well as the fact that the agent has no myelosuppressive toxicity, suggests that spirogermanium should be further investigated, both alone and in combination with other agents.

REFERENCES

1. RICE LM, WHEELER JW, and GESCHICKTER CF. Spirans XXII. Synthesis of 4.4-dialkyl-4-germacyclohecanone and 8.8-dialkyl-8-germaazospiro (4.5) decanes. J Heterocycl Chem 11:1041–1074, 1974.
2. SCHEIN PS, SLAVIK M, and SMYTHE T, ET AL. Phase I clinical trial of spirogermanium. Cancer Treat Rep 64:1051–1056, 1980.
3. MATTSSON W, TROPE C, and ORBERT B. A phase I study of spirogermanium. Proc Am Assoc Cancer Res and ASCO 21:778, 1980.
4. BUDMAN DR, SCHULMAN P, VINCIGUERRA V, ET AL. Phase I trial of spirogermanium given by infusion in a multiple-dose schedule. Cancer Treat Rep 66:173–175, 1982.

Inefficacy of Sc *Corynebacterium parvum* in Stage I Malignant Melanoma: Preliminary Results of a Single-Institution Pilot Study[1,2]

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Previous trials using *Corynebacterium parvum* as an adjuvant to surgery in stage II and stage III malignant melanoma have been disappointing (1–3). To date, there has been only one published randomized trial which compares surgery plus *C. parvum* to surgery alone in stage I disease (3).

From March 1975 until December 1981, 34 patients with stage I malignant melanoma started receiving sc injections of *C. parvum* (Burroughs Wellcome, Research Triangle Park, NC) within 3 weeks following surgery at our institution. Patients received 4 mg/m^2 of *C. parvum* weekly for 6 weeks, monthly for 6 months,

TABLE 1.—Characteristics of patients treated with surgery + *C. parvum* vs surgery alone

Characteristic	No. of patients (%)		
	Surgery + *C. parvum* (34 patients)*	Surgery alone (63 patients)†	*P* value‡
Male	19 (56)	38 (65)	0.41
Female	15 (44)	21 (35)	0.42
Site of primary			
Extremity	12 (38)	8 (20)	0.10
Trunk	13 (40)	27 (68)	0.03
Head and neck	7 (23)	5 (13)	0.31
Clark's level of invasion			
III	18 (53)	4 (9)	0.001
IV	11 (32)	32 (70)	0.001
V	5 (15)	10 (21)	0.41
In remission at 5 yrs	24 (71)	47 (74)	0.43
Surviving at 5 yrs	28 (82)	55 (87)	0.47

*Mean age: 47 yrs in both groups.

†*Note:* Some of the initial data with respect to sex, site, and Clark's level were incomplete in the surgery-alone group. Hence, the total No. of patients analyzed with respect to these characteristics does not always total 63.

‡Sex, primary site, and level of invasion comparison by chi-square analysis; remission duration and survival by Kaplan-Meier product-limit method and Gehan's generalized Wilcoxon test, respectively.

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and then every 6 months up to 5 years or relapse. Remission duration and survival were compared to those in a group of untreated concurrent controls obtained by special permission from the Southwest Oncology Group.

Patient characteristics are listed in table 1. Groups were not comparable with respect to Clark's level and site; 53% of treated patients had Clark's level III lesions compared to only 9% of the surgery-alone group ($P < 0.001$; chi-square analysis). The untreated group had a higher percentage of patients with Clark's level IV tumors. For remission duration and survival analysis, Clark's levels III and IV were combined in each group. There were more trunk lesions in the control group.

There were no significant differences in overall remission duration or survival between groups. At 5 years, ten patients treated with *C. parvum* had relapsed compared to 16 in the surgery-alone group ($P = 0.43$). There were six deaths in the *C. parvum* group versus eight deaths in the control group ($P = 0.47$). There was no difference in either parameter with respect to age, sex, site, or Clark's level. Side effects of *C. parvum* were minimal and consisted of low-grade fever and mild inflammation at the injection site.

Although this study was not randomized and contained a small number of patients, our results are similar to a recent multicenter trial by Balch et al (3). Despite the larger number of Clark's level III patients and extremity primaries in the treated group, a survival advantage was not demonstrated. However, overall thickness as measured in millimeters may be of greater prognostic importance than Clark's level (4,5). Although preliminary, these results to date suggest that *C. parvum* administered in this fashion offers no significant benefit over surgery alone in stage I melanoma.

REFERENCES

1. Elias YH, Pinsky CM, Hirshaut Y, et al. Surgical adjuvant therapy of malignant melanoma with *Corynebacterium parvum*. Cancer 48:245–251, 1981.
2. Presant CA, Bartolucci AA, Smalley RV, et al. Effect of *Corynebacterium parvum* on combination chemotherapy of disseminated malignant melanoma. *In* Immunotherapy of Cancer: present Status of Trials in Man (Terry WD, and Windhorst D, eds). New York, Raven Press, pp 113–121.
3. Balch CM, Smalley RV, Bartolucci AA, et al. A randomized prospective trial of adjuvant *C. parvum* immunotherapy in 260 patients with clinically localized melanoma (stage I). Prognostic factors and preliminary results of immunotherapy. Cancer 49:1079–1084, 1982.
4. Breslow A. Thickness, cross sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg 172:902–908, 1972.
5. Balch CM, Murad TM, Soong SJ, et al. A multifactional analysis of melanoma: I. Prognostic histopathological features comparing Clark's and Breslow's staging methods. Ann Surg 188:732–742, 1978.

Phase II Evaluation of Spirogermanium in Advanced Ovarian Carcinoma[1]

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Spirogermanium is an azaspiran discovered in 1974 by Rice et al (1). The compound consists of a germanium substitution for one carbon moiety in a heterocyclic ring structure. In vitro activity was found against HeLa, L1210 leukemia, and human malignant lymphoma cells (2). An initial report of activity in advanced ovarian carcinoma (3) stimulated our interest in this new drug that had limited myelotoxicity. Phase I data suggested a starting dose of 50–80 mg/m^2 iv every other day for 4 weeks (4).

TABLE 1.—Patient characteristics

Characteristic	No. of patients (total = 17)
Age (yrs)	
Median: 55	
Range: 28–76	
Median Karnofsky performance score (range): 80% (90%–30%)	
Previous chemotherapy	16 (94%)
1 protocol	6
≥ 2 protocols	10
Previous radiation therapy	5 (29%)
Abdominal	3
Pelvic	2
Toxic effect	
Slight-to-moderate nausea and vomiting	9 (53%)
Blurred vision	5 (29%)
Fatigue	4 (24%)
Lethargy	3 (18%)
Lightheadedness	3 (18%)
Phlebitis at injection site	2 (12%)
Metallic taste in mouth	1 (6%)

Seventeen evaluable patients with advanced, previously treated ovarian carcinoma were given spirogermanium at a starting dose of 80 mg/m^2 by iv drip over 30 minutes every other day. In some patients, the dose was escalated to 120 mg/m^2, based on toxicity. All patients except one with a granulosa cell tumor had epithelial ovarian carcinoma. Characteristics of the patients are listed in table 1. All patients except one were previously treated with chemotherapy consisting of various combinations of hexamethylmelamine, cyclophosphamide, doxorubicin, cisplatin, 5-FU, and melphalan. Three patients had received prior treatment with whole-abdominal radiation therapy, and two patients had been treated with whole-pelvis irradiation. All patients had measurable disease as documented by pelvic examination, lymphadenopathy, chest radiography, or computed tomography. The development of increased amounts of ascitic fluid was considered to show failure of treatment. Standard criteria were used to assess response.

No patient responded to treatment. An adequate trial consisted of a minimum of 2 weeks of treatment (six administered doses). Patients were treated for at least 4 weeks unless there was rapid tumor progression or severe drug toxicity. The mean total dose administered was 1530 mg (range, 575–3048) over a median treatment period of 4 weeks (range, 1–16). Median survival following treatment was 72 days (range, 13–403+). The drug was well-tolerated. No myelosuppression occurred at the doses administered. Major toxic effects are listed in table 1. One patient developed severe, intractable nausea and vomiting following drug administration. When the drug was discontinued, the symptoms improved. The patient refused further treatment after five drug doses.

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Our experience with spirogermanium in the heavily pretreated patients with advanced disease differs from the recently published experience of Tropé et al (3). In spite of the higher doses administered, no responses were observed in our group of patients. At a dose of 80–120 mg/m^2, we observed gastrointestinal side effects not previously described (3). Most toxic effects were modest and easily tolerated. We conclude that spirogermanium does not have activity (< 20% response rate with 95% confidence) in patients with previously treated ovarian carcinoma.

REFERENCES

1. Rice LM, Wheeler JW, and Geschickter CF. Spirans-synthesis of 4,4-dialkyl-4-germacyclohexanone and 8,8-dialkyl-8-germazospiro (4.5) dicane. J Heterocyclic Chem 11:1041–1047, 1974.
2. A phase II study on the effects of spirogermanium hydrochloride. *In* Master Guide. Somerville, NJ, Unimed, Inc, 1981.
3. Tropé C, Mattsson W, Gynning I, et al. Phase II study of spirogermanium in advanced ovarian malignancy. Cancer Treat Rep 65:119–120, 1981.
4. Schein PS, Slavik M, Smythe T, et al. Phase I clinical trial of spirogermanium. Cancer Treat Rep 64:1051–1056, 1980.

Phase II Clinical Study of Yoshi 864 in Squamous Cell Carcinoma of the Uterine Cervix[1,2]

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For the Gynecologic Oncology Group

Yoshi 864, a busulfan analog, is active against experimental tumors resistant to nitrogen mustards (1). This drug was reported to have some activity against squamous cell carcinoma of the uterine cervix in a broad phase II study at dose causing minimal toxicity (2). Therefore, a group-wide phase II study of Yoshi 864 in squamous cell cervical carcinoma, using the same schedule, was undertaken. Eighteen patients with advanced or recurrent, histologically confirmed squamous cell carcinoma of the uterine cervix not amenable to further surgery, radiotherapy, or higher priority chemotherapy were evaluated. All patients had measurable disease. Neither ascites nor pleural effusion alone was considered measurable for purposes of this study. Sonography was accepted if the lesion was sharply defined during initial examination. All patients had a Gynecologic Oncology Group performance status ≤ 3 (median, 2). Patients must have passed their nadir blood cell counts from previous therapy and have a granulocyte count $\geq 1500/mm^3$ and a platelet count $\geq 100{,}000/mm^3$ before study entry. A BUN < 25 mg/100 ml, serum creatinine < 1.5 mg/100 ml, bilirubin < 1.0 mg/100 ml, and SGOT < 50 IU/L were required. Standard response criteria were used (3). Complete response was defined as the disappearance of all gross evidence of disease for at least 1 month; partial response was defined as a $\geq$ 50% reduction in the sum of the products of the largest diameter and the perpendicular of all measurable lesions; progressive disease was defined as a $\geq 50\%$ increase in the above-mentioned product or the appearance of any new lesions within 3 months of study; and stable disease was defined as a response not meeting criteria for complete or partial response or progressive disease.

Initially, Yoshi 864 was administered by a slow iv push at a dose of 2.0 mg/kg/day for 5 days, repeated every 6 weeks. Dose adjustment to 1.5 mg/kg/day was required when grade 2 bone marrow toxicity occurred in the previous course. Subsequently, the starting dose of Yoshi 864 was decreased to 1.5 mg/kg/day because of the severe bone marrow toxicity observed in patients with epithelial ovarian cancer who were given 2.0 mg/kg/day of Yoshi 864 in our preceding phase II clinical study (4).

Patient characteristics, response to therapy, and toxic effects are summarized in table 1. No objective tumor regressions occurred. Of the 18 patients, four had stable disease and 14 had progressive disease. In three patients the most serious toxic effect was thrombocytopenia, with a median platelet count nadir of $65{,}000/mm^3$ (range, 61,000–144,000), which occurred at median

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TABLE 1.—Patient characteristics

Age (yrs)	Site of disease*	Gynecologic Oncology Group performance status	Histologic grade	Previous treatment†	Response‡	No. of courses	Highest dose (mg/kg)	Toxic effects§
40	P	2	3	RT, CIS	ID	1	2.0	—
41	E	3	1	RT, CIS	ID	1	1.5	Anemia, diffuse encephalopathy
42	E	1	2	RT, CIS	ID	2	2.0	Nausea, vomiting
43	E	2	3	RT, CIS	ID	1	1.5	Leukopenia (3100)
44	P	0	3	RT, CIS	ID	1	2.0	—
44	P	3	2	RT, *C. parvum*, CIS, ADR	ID	1	2.0	Fever
45	E	2	3	RT, CIS	ID	1	2.0	—
52	E	0	1	RT, MMC, CIS	ID	3	2.5	Leukopenia (2700), nausea, vomiting
52	E	1	3	RT, BLEO, CIS-MTX-VCR	SD	5	1.5	—
52	P	3	2	RT, CIS-CTX	ID	1	2.0	—
54	P	2	2	RT, CIS	SD	3	1.5	Leukopenia (2600), thrombocytopenia (144,000), nausea, vomiting, numbness
54	P	2	2	RT, CIS-CTX	SD	3	2.0	Thrombocytopenia (65,000)
55	E	2	3	RT, CIS, BLEO-MMC	ID	3	1.5	—
55	E	2	Unknown	RT, CIS	ID	3	2.0	Nausea, vomiting
56	E	3	1	RT, CIS-CTX	ID	2	2.0	—
60	P	0	2	RT, CIS	ID	2	1.5	—
63	P	1	2	RT, CIS	ID	2	2.0	Hypotension, fever
67	P	1	2	RT, CIS	SD	3	1.5	Anemia, Hgb of 6.1 g/100 ml; thrombocytopenia (61,000)

*P = pelvic; E = extrapelvic.

†RT = radiation therapy; CIS = cisplatin; *C. parvum* = *Corynebacterium parvum;* ADR = doxorubicin; MMC = mitomycin; BLEO = bleomycin; MTX = methotrexate; VCR = vincristine; CTX = cyclophosphamide.

‡ID = increasing disease; SD = stable disease.

§Values in parentheses = cells/mm^3.

Course 3. Leukopenia, with a median wbc count nadir of 2700/mm^3 (range, 2600–3100), was reported in three patients after median Course 3 (range, 1–3). Neurotoxicity, which presented as comatose state or paresthesias, developed in two patients. However, the patient who developed coma also had CNS metastases. The other toxic effects included nausea and vomiting in four patients and fever and hypotension in individual cases.

We conclude that Yoshi 864 at this dose schedule is not an active drug in the treatment of patients with advanced squamous cell carcinoma of the uterine cervix recurring after previous treatment with chemotherapy.

REFERENCES

1. EL MERZABANI MN, and SAKURAI Y. A new alkylating antitumor agent effective on experimental tumors resistant to nitrogen mustard. Gan 56:589–598, 1965.
2. ALTMAN SJ, METTER GE, NEALON TF, ET AL. Yoshi 864 (1-propanol, 3,3′-iminodi-, dimethanesulfonate [ester], hydrochloride): a phase II study in solid tumors. Cancer Treat Rep 62:389–395, 1978.
3. SLAYTON RE, CREASMAN WT, PETTY W, ET AL. Phase II trial of VP-16-213 in the treatment of advanced squamous cell carcinoma of the cervix and adenocarcinoma of the ovary: a Gynecologic Oncology Group study. Cancer Treat Rep 63:2089–2092, 1979.
4. SLAVIK M, MUSS H, BLESSING JA, ET AL. Phase II clinical study of Yoshi 864 in epithelial ovarian carcinoma: a Gynecologic Oncology Group study. Cancer Treat Rep 66:1775–1777, 1982.

Phase II Trial of AMSA in Patients With Advanced or Recurrent Squamous Cell Carcinoma of the Cervix: A Gynecologic Oncology Group Study[1,2]

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AMSA is an acridine derivative that has shown antitumor activity in a number of animal tumors (1). The drug inhibits DNA synthesis through intercalation, while having little effect on RNA synthesis (1). Remission of human leukemia has been achieved with AMSA (2).

From August 1979 to January 1982, the Gynecologic Oncology Group (GOG) conducted a phase II trial of AMSA in patients with recurrent or advanced squamous cell carcinoma of the cervix. A dose of 90 mg/m^2 of the drug was diluted with 500 ml of 5% dextrose in water and infused iv over 1 hour. Because three of the first 11 patients had severe leukopenia after one course of treatment, the dose was modified to 60 mg/m^2. Treatment was repeated every 21 days if the patient had recovered from the toxic effects of the previous course.

Responses, performance status, and grades of toxicity were defined according to previously described GOG criteria (3).

Patient characteristics, response to therapy, and toxic effects are summarized in table 1. Twenty-seven patients gave informed consent, but two withdrew from the protocol prior to receiving the first treatment. Responses in the 25 evaluable patients were as follows: one patient who had not previously received chemotherapy had complete remission of pelvic tumor lasting 11 months, four patients had stable disease, and 20 patients had progressive disease. Brenner et al (4) have reported similar results with higher doses of AMSA in patients with squamous cell carcinoma of the cervix.

Although leukopenia was the major toxic effect, it was generally not severe, with a median wbc count nadir of 2900/mm^3. However, there appears to be a subset of patients who are particularly sensitive to the myelosuppressive effect of AMSA. Among 11 patients treated with one course at 90 mg/m^2, two patients had grade 4 leukopenia and one had grade 3 leukopenia. Other investigators have observed severe myelosuppression in a small percentage of patients receiving AMSA (5). One case of sudden death occurred several hours after infusion of AMSA; neither an electrocardiogram nor an autopsy was performed.

We conclude that AMSA at the doses and schedule utilized in this trial has negligible activity in patients with cervical carcinoma previously treated with radiation and chemotherapy.

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TABLE 1.—Patient characteristics

Age (yrs)	GOG performance status	Previous treatment*	Response†	No. of courses	Toxic effects‡
29	1	RT, CIS	ID	2	Nausea, vomiting
31	1	RT, VCR-BLEO-MITO	ID	2	None
32	2	RT, CIS	ID	2	Leukopenia (1400)
33	0	RT, CIS-CTX	ID	1	None
33	3	RT, hydroxyurea	CR	3	Nausea, vomiting, anemia, vesicovaginal fistula
37	1	RT, CIS	ID	2	Leukopenia (100)
37	1	RT, ADR-CIS	SD	4	Nausea
44	0	RT, ADR-CTX, CIS-CTX	ID	2	None
46	1	RT, CTX-MTX, CIS	ID	2	Leukopenia (3800)
49	2	RT, VCR	ID	1	None
51	1	RT, CIS	ID	4	Nausea, vomiting, leukopenia (3000)
51	1	RT-CIS, BLEO	SD	2	Leukopenia (3200), thrombocytopenia (50,000)
51	2	RT, BLEO-CIS	NC	5	Leukopenia (3000), thrombocytopenia (117,000), nausea
52	2	RT, CIS	SD	2	None
53	3	RT, CIS, BLEO-CTX, MITO	NA	1	Sudden death
54	2	RT, CIS	ID	2	Nausea, vomiting, lethargy
54	2	RT, *C. parvum*, CIS, VCR	ID	2	None
55	2	CIS	ID	1	None
55	3	RT, CIS	ID	1	Nausea
56	1	RT, CIS	ID	1	None
61	1	RT, CIS	ID	1	Leukopenia (3600)
65	1	RT, CIS	ID	2	Nausea
70	3	RT, CIS	SD	3	Leukopenia (500)
78	2	RT, CIS	ID	2	None
79	2	RT, CIS	ID	3	Nausea, vomiting, leukopenia (2900)

*RT = radiation therapy; CIS = cisplatin; VCR = vincristine; BLEO = bleomycin; MITO = mitomycin; CTX = cyclophosphamide; ADR = doxorubicin; MTX = methotrexate; *C. parvum* = *Corynebacterium parvum*.

†ID = increasing disease; CR = complete response; SD = stable disease; NC = no change; NA = not applicable.

‡Values in parentheses = cells/mm^3.

REFERENCES

1. WEISS RB, CHARLES LM, JR, and MACDONALD JS. *m*-AMSA: an exciting new drug in the National Cancer Institute Drug Development Program. Cancer Clin Trials 3:203–209, 1980.
2. LAWRENCE HJ, RIES CA, REYNOLDS RD, ET AL. AMSA—a promising new agent in refractory acute leukemia. Cancer Treat Rep 66:1475–1478, 1982.
3. THIGPEN T, SHINGLETON H, HOMESLEY H, ET AL. *cis*-Dicalorodiammineplatinum(II) in the treatment of gynecologic malignancies: phase II trials by the Gynecologic Oncology Group. Cancer Treat Rep 63:1549–1555, 1979.
4. BRENNER DE, GARBINO C, KASDORF H, ET AL. A phase II trial of AMSA in the treatment of advanced gynecologic malignancies. Am J Clin Oncol 5:291–295, 1982.
5. SAMSON MK, FRAILE RJ, BAKER LH, ET AL. Phase II study of AMSA in lung cancer. Cancer Treat Rep 65:655–658, 1981.

Full-Thickness Skin Necrosis Due to Inadvertent Interstitial Infusion of Cisplatin

A patient is described who had a previously unreported complication of cisplatin: full-thickness skin necrosis following inadvertent subcutaneous infusion of cisplatin into the dorsum of the left hand.

Workers exposed to platinum salts may develop an illness characterized by cutaneous (erythema, pruritus, and urticaria) manifestations, among other problems (1), but there is only one previous case report of a skin reaction due to inadvertent extravasation of cisplatin (2).

A 67-year-old woman was found to have stage IV carcinoma of the ovary. Treatment included a total abdominal hysterectomy and bilateral salpingo-oophorectomy, omentectomy, and bulk reduction surgery.

She was randomized in the Clinical Oncology Society of Australia/Ludwig Institute ovarian carcinoma chemotherapy trial to receive chlorambucil for 14 days followed by a single infusion of cisplatin (50 mg/m^2). This treatment was carried out for 2 weeks every month. When the patient was hospitalized for the second dose of cisplatin, an iv line was inserted for prehydration; the following day, cisplatin at a dose of 75 mg in 100 ml of normal saline was infused via the iv line sited in the dorsum of the left hand. This infusion was to take place over 60 minutes. The patient, unfortunately, had become confused following the initial surgery; because of her confused state there had been considerable difficulty in maintaining iv infusions, since she was in the habit of adjusting and removing them. Although restraining braces were used on the limbs, there was extravasation of the iv fluid into the interstitial space at the time of the second cisplatin dose, and most of the dose was infused into the dorsum of the hand. When this was recognized, the iv line was removed and the arm was elevated. Subsequently, pain, swelling, and signs of inflammation developed over a 2-week period. There was no treatment with local injections of corticosteroids. A large area of necrosis on the dorsum of the left hand developed. Necrosis involved the skin, subcutaneous fat, and extensor tendons. There was considerable undermining of skin edges, since the fat necrosis was more extensive than the overlying skin loss. Ulceration extended from the wrist to the metacarpophalangeal joints and across the breadth of the dorsum of the hand. Treatment with antibiotics, eusol dressings, immobilization of the hand in a volar plaster, and elevation of the hand was followed 1 week later by radical debridement of all necrotic tissue. After 1 additional week the defect was covered with split skin grafts and, after subsequent debridement, secondary grafting, and elaborate plastering and dressing, the area eventually healed.

It is well-known that a variety of cytotoxic drugs can cause tissue necrosis if given into the subcutaneous tissue following extravasation due to poor positioning of the iv catheter in the first instance or the displacement of a catheter during the infusion. It is therefore advised that fresh iv sites be used for any such injections. Subcutaneous tissue necrosis associated with cytotoxic agents and occurring in areas with little subcutaneous fat (ie, dorsum of the hands) can result in skin necrosis in addition to subcutaneous tissue necrosis. Because of this, it is usually recommended that the dorsum of the hand not be used for the injection of these cytotoxic agents. Nevertheless, since these agents are toxic to veins, it is common for other suitable iv sites to be unsuitable, particularly in children and women, and as a result, the dorsum of the hand has to be used. In patients who are uncooperative, confused, or demented it is recommended that this particular iv site not be used. Patients should be restrained if supervision is inadequate. Bolus or push injections could be given as an alternative, bearing in mind that the pharmacokinetics of the drug may differ in this circumstance, leading possibly to different side effects (3,4).

REFERENCES

1. Parrot JL, Hebert R, Saindelle A, et al. Platinum and platinosis. Allergy and histamine release due to some platinum salts. Arch Environ Health 19:685–691, 1963.
2. Lewis KP, and Medina WD. Cellulitis and fibrosis due to *cis*-diamminedichloroplatinum(II) (Platinol) infiltration. Cancer Treat Rep 64:1162–1163, 1980.
3. Jacobs C, Bertino JR, Goffinet DR, et al. 24 Hour infusion of *cis*-platinum in head and neck cancers. Cancer 42:2135–2140, 1978.
4. Salem P, Hall SW, Benjamin RS, et al. Clinical phase I–II study of *cis*-dichlorodiammineplatinum(II) given by continuous iv infusion. Cancer Treat Rep 62:1553–1555, 1978.

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Induction Chemotherapy With Cisplatin and Cyclophosphamide and Maintenance Chemotherapy With Doxorubicin and 5-FU in Advanced Urinary Bladder Tumors

Between October 1977 and August 1981, 12 patients with metastatic transitional cell carcinoma of the urinary bladder, one with metastatic carcinoma of the ureter, and one with metastatic carcinoma of the renal pelvis were treated with cisplatin, cyclophosphamide, doxorubicin, and 5-FU (CCAF) given in conventional doses, but in a sequential and alternating manner in an attempt to improve response rate and decrease toxicity.

There were 12 males and two females, with a median age of 66 years (range, 42–76). Nine patients had undergone major surgery and 13 had received prior radiation therapy to the pelvic area, but none had been given prior systemic chemotherapy. The group had a median Karnofsky performance status of 60% (range, 40%–80%). Most frequent sites with measurable metastatic disease were the lungs (six patients), liver (five), bones (four), and soft tissues (two). A pelvic mass provided a single parameter to evaluate response in only one patient. The median interval from diagnosis of metastases to the start of chemotherapy was 1 month (range, 0.5–10).

The chemotherapeutic regimen is shown in table 1. The induction phase consisted of cisplatin and cyclophosphamide given iv in 120 ml of normal saline over 15–20 minutes each to hospitalized patients under iv hydration until chemotherapy-induced vomiting subsided. Maintenance therapy with doxorubicin and 5-FU was delivered on an outpatient basis.

Three of the 14 patients (21%) achieved a partial remission (PR), with a ≥ 50% decrease of measurable lesions lasting 8, 7, and 3 months; responses were seen in the liver, lungs, and skeleton, respectively. One patient (8%) with a pelvic mass achieved improvement (25%–50% decrease of measurable disease) for 5 months, and three patients (21%) had stabilization of disease for 9 (lungs), 4+ (liver, vagina), and 2 (liver) months, respectively. Seven patients (50%) had disease progression while receiving CCAF.

All patients achieving either PR or disease improvement had primary bladder tumors; the rate of PR plus improvement in this subgroup of 12 patients was 33%. The patient with renal pelvis carcinoma had stable lung metastasis for 9 months and the patient with ureter carcinoma failed to respond to chemotherapy.

Age, performance status, interval from diagnosis of the primary tumor to the detection of metastases, and degree of myelosuppression did not correlate with response to chemotherapy, but in three of four patients achieving PR or disease improvement metastases were

TABLE 1.—CCAF: scheme and schedule of treatment

A. Induction regimen
Cisplatin (60 mg/m^2 iv, Day 1)
Cyclophosphamide (600 mg/m^2 iv, Day 1)

Followed 3 wks later by "B."

B. Maintenance regimen
Doxorubicin (60 mg/m^2 iv, Day 1)
5-FU (600 mg/m^2 iv, Days 1 and 8)

Every 3 wks for 3 courses, then "C."

C. Reinduction as in "A," followed by maintenance in "B," as illustrated below:

Wk No.	Cisplatin	Cyclophosphamide	Doxorubicin	5-FU
1	X	X		
3			X	X
4				X
6			X	X
7				X
9			X	X
10				X
12	X	X		
15			X	X
16				X
18			X	X
19				X
21			X	X
22				X
24	X	X		
Etc				

limited to one organ while five of seven with disease progression had two to four metastatic sites.

Median survival from the start of chemotherapy was 4.5 months (range, 1.5–20) for the whole group, increasing to 11 months (range, 4–20) in patients achieving PR or improvement, but was only 4 months for patients with either stable disease (range, 3–11 months) or disease progression (range, 1.5–8.5 months), and the difference with responders is significant ($P < 0.05$, Mantel-Cox test).

The mean given doses were close to the calculated doses for all four drugs. Nausea and vomiting (moderate to severe) and alopecia were universal. While blood cell counts in this study do not necessarily reflect maximal myelosuppression, the median wbc count nadir was 3000/mm^3 (range, 900–6400) and the median platelet count nadir was 112,000/mm^3 (range, 76,000–376,000). One granulocytopenic patient developed cellulitis of the lower abdominal wall in a previously irradiated area; anemia < 8 g/100 ml was observed in three patients. There were no bleeding episodes; hemorrhagic cystitis, clinically detectable hearing loss, and doxorubicin-induced cardiomyopathy were not observed. While all patients had a mild elevation of BUN, 44% experienced an increase in serum creatinine (median increase, 1.6 mg/100 ml; range, 1–3.4), and 71% had a transient drop in creatinine clearance (median decrease, 24 ml/minute from pretreatment values; range, 9–60); one patient who had received three induction courses with cisplatin and cyclophosphamide had an irreversible decrease in creatinine clearance to 25 ml/minute.

DISCUSSION

The regimen utilized in the present phase II study includes the most active known agents in metastatic bladder tumors. As opposed to other trials, chemotherapy was administered sequentially with cisplatin and cyclophosphamide given initially in an attempt to induce prompt tumor regression as reported by others (1). However, courses were repeated at relatively long intervals to avoid the limiting factor of severe, persistent nausea and vomiting and thus to assure patient compliance. Maintenance therapy with doxorubicin and 5-FU was selected in view of their activity, as single agents as well as in combination, in bladder tumors (2,3).

Our study further establishes that urothelial transitional cell carcinomas are responsive to chemotherapy, with tumor regression seen in both visceral and skeletal metastases. Furthermore, responders lived significantly longer than nonresponders. Although responses were evident within 2 months from the start of chemotherapy, cisplatin and cyclophosphamide failed to induce remission in most patients and neither the sequential administration of doxorubicin and 5-FU nor the reinduction courses with cisplatin and cyclophosphamide improved our low initial response rate. Thus, our overall results (a 21% PR rate and an 8% improvement rate) are disappointingly lower than recently published trials with three-drug regimens including cisplatin, doxorubicin, and either cyclophosphamide or 5-FU, in which objective responses were achieved in 48%–90% of the patients, including a few complete remissions (4–7).

In these studies, and differing importantly from our protocol design, cisplatin was given at short intervals. This seems to explain the difference in results and would indicate that cisplatin given intermittently at long intervals, as we did, is not useful in urothelial tumors.

Although our protocol was fairly well-tolerated and required only 2 in-hospital days every 3 months, it would appear that the use of more aggressive combination chemotherapy schemes in higher, more frequent dose schedules is required to increase response rate and prolong survival in patients with transitional cell tumors of the urinary tract.

REFERENCES

1. Yagoda A. Future implications of phase II chemotherapy trials in ninety-five patients with measurable advanced bladder cancer. Cancer Res 37:2775–2780, 1977.
2. Carter SK, and Wasserman TH. The chemotherapy of urologic cancer. Cancer 36:729–747, 1975.
3. Cross RJ, Glashan RW, Humphrey CS, et al. Treatment of advanced bladder cancer with adriamycin and 5-fluorouracil. Br J Urol 48:609–615, 1976.
4. Yagoda A. Phase II trials with *cis*-dichlorodiammineplatinum(II) in the treatment of urothelial cancer. Cancer Treat Rep 63:1565–1572, 1979.
5. Williams SD, Donohue JP, and Einhorn LH. Advanced bladder cancer: therapy with *cis*-dichlorodiammineplatinum(II), adriamycin and 5-fluorouracil. Cancer Treat Rep 63:1573–1576, 1979.
6. Kedia KR, Gibbons C, and Persky L. The management of advanced bladder carcinoma. J Urol 125:655–658, 1981.
7. Sternberg JJ, Bracken RB, Handel PB, et al. Combination chemotherapy (CISCA) for advanced urinary tract carcinoma. A preliminary report. JAMA 238:2282–2287, 1977.

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Is Further Intensification of Treatment Warranted in Acute Nonlymphoblastic Leukemia?

Recent articles by Mayer et al (1) and Preisler (2) support the concept that intensive postinduction chemotherapy offers the best chance for cure of acute nonlymphoblastic leukemia. Both papers rightly point out that most "maintenance" phase chemotherapy programs are inadequate and, indeed, do not serve to eradicate the malignant clone. It is claimed that by intensifying the use of currently available drugs that have activity against leukemia, a cure is possible in an increasing number of patients. Our own experience with this approach suggests that this recommendation must be approached with caution. The L-14 protocol (3) was devised with curative intent. The first patient began treatment in August 1977, and entry onto the protocol was stopped in April 1979. Remission induction was accomplished with daunorubicin, cytarabine (ara-C), and thioguanine in 64% of 53 consecutive previously untreated patients. All remissions were achieved within one course, and analysis revealed that cell killing was more rapid with this regimen than with a somewhat different induction program (4). After remission, the patients received repeated infusions of ara-C with daunorubicin and thioguanine, hydroxyurea, and methotrexate for 14 months, and the courses were interrupted periodically with carmustine and cyclophosphamide in an effort to eliminate G_0 cells not affected by the primarily cycle-specific chemotherapy. The ara-C infusions were administered with intent to induce cytopenia, which occurred in virtually all instances, thus fulfilling the criteria of consolidation therapy mentioned by both Mayer and Preisler. Of the 34 patients who achieved remission and continued to the intensification schedule, eight died while still in remission. Of equal significance was that of those patients who were able to tolerate this eradication schedule, 23 have relapsed and only three are currently in remission. Our study points to the inability of the available chemotherapeutic agents (when given in maximum doses) to eradicate leukemia in most patients. The observation by Mayer et al (1) that the patients who had the longest survival have subsequently relapsed indicates that further relapses will occur as the patients who receive VAPA get farther along in their off-therapy phase. These comments are not intended to discourage efforts directed at cure of this disease but to emphasize that there are severe limits placed on us all by the drugs that are available. They suggest that until more active agents are discovered and incorporated into the type of program described by Mayer et al, other avenues have to be explored. In this regard, there is an ongoing need to improve the methods currently used in transplantation as well as to develop innovative approaches such as the use of noncytotoxic antileukemic agents (5).

REFERENCES

1. Mayer RJ, Weinstein HJ, Coral FS, et al. The role of intensive postinduction chemotherapy in the management of patients with acute myelogenous leukemia. Cancer Treat Rep 66:1455–1462, 1982.
2. Preisler HD. Therapy for patients with acute myelocytic leukemia who enter remission: bone marrow transplantation or chemotherapy? Cancer Treat Rep 66:1467–1473, 1982.
3. Arlin Z, Gee T, Fried J, et al. Rapid induction of remission in acute non-lymphocytic leukemia (ANLL). Proc Am Assoc Cancer Res and ASCO 20:112, 1979.
4. Hiddemann W, Andreeff M, Buchner T, et al. Quantitation of therapy induced cytoreduction in acute myeloblastic leukemia (AML): a comparison of two induction regimens. Proc Am Assoc Cancer Res 23:118, 1982.
5. Breitman TR, Collins ST, and Keene BR. Terminal differentiation of human promyelocytic leukemia cells in primary culture in response to retinoic acid. Blood 57:1000–1004, 1981.

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Reply

The concern expressed by Arlin et al about the inability of available chemotherapeutic agents to totally eradicate acute myelogenous leukemia (AML) in most patients is certainly appropriate. Because of differences in experimental design, however, it is unclear whether the results of the L-14 protocol of Memorial Hospital

should be compared to the more encouraging data reported from the Sidney Farber Cancer Institute (1), the Roswell Park Memorial Institute (2), and St. Bartholomew's Hospital (3). Following the attainment of remission, patients given the L-14 regimen received 14 months of postremission therapy including such drugs as carmustine, cyclophosphamide, and hydroxyurea, which are relatively inactive in the treatment of AML. While these patients were also given infusions with cytarabine (ara-C), the dose schedule of such treatment was most likely less intensive than that used during induction, since myelosuppression was apparently not always observed. This therapeutic approach differs from the intensive consolidation incorporated into the treatment regimens at the other three institutions. Such intensive postremission chemotherapy is directed at the maximal cytoreduction of residual leukemic cells through the administration of the most active chemotherapeutic agents at dose schedules equal to or greater than those given during induction. This concept is based on data in experimental tumors (4) and in human leukemic cells in short-term tissue culture (5) demonstrating a steep dose (concentration)-response curve for ara-C.

Further improvement in the treatment of patients with AML will require the optimal application of all effective therapeutic modalities. Dose intensification with ara-C appears to lead to a prolonged duration of remission and possibly to a higher rate of cure. The promising results with this therapeutic approach warrant its definitive evaluation through randomized comparison with a more conventional postremission treatment plan.

REFERENCES

1. Mayer RJ, Weinstein HJ, Coral FS, et al. The role of intensive postinduction chemotherapy in the management of patients with acute myelogenous leukemia. Cancer Treat Rep 66:1455–1462, 1982.
2. Preisler HD. Therapy for patients with acute myelocytic leukemia who enter remission: bone marrow transplantation or chemotherapy? Cancer Treat Rep 66:1467–1473, 1982.
3. Bell R, Rohatiner AZS, Clevin ML, et al. Short-term treatment for acute myelogenous leukemia. Br Med J 284:1221–1224, 1982.
4. Skipper HE. Reasons for success and failure in treatment of murine leukemia with drugs now employed in treating human leukemias. *In* Cancer Chemotherapy, Ann Arbor, Monograph Publishers-University Microfilms International, 1978, vol 1, pp 1–166.
5. McCulloch EA, Buick RN, Curtis JE, et al. The heritable nature of clonal characteristics in acute myeloblastic leukemia. Blood 58:105–109, 1981.

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Reply

Arlin and co-workers pose the question: Is further intensification of treatment warranted in acute nonlymphoblastic leukemia (ANLL)? They conclude, based on their own experience, that the available chemotherapeutic agents (when given in maximum doses) are unable to eradicate leukemia in most patients. While the conclusions of Arlin et al may prove to be correct, the investigators do not provide data to substantiate their conclusions. An analysis of their letter using the guidelines proposed in the paper to which they refer (1) will explain why their conclusions regarding their own study cannot be used to draw principles regarding the efficacy or nonefficacy of intensive consolidation chemotherapy as an approach to treat patients with ANLL.

The authors report the results of an "intensive" consolidation regimen that was employed at Memorial Hospital. Of the patients receiving this treatment, 24% died in remission, and the regimen did not prevent most of the surviving patients from relapsing. It is stated that the regimen was intensive since "cytopenia" was produced. The following two fundamental questions must be asked about the study.

(*a*) *Did Arlin et al employ an "intensive" regimen?*—One cannot answer this question since the investigators do not provide information regarding the depth and duration of the pancytopenia produced by their regimen, the number of episodes of pancytopenia each patient experienced, or how often pancytopenia was induced. To simply state that "cytopenia" was produced does not provide sufficient information to permit evaluation of the authors' contention that the consolidation regimen was "intensive." The absence of such information prevents any comparison to be made between the "intensity" of the Memorial Hospital regimen and the intensity of any other regimen reported in the literature.

(*b*) *Did the Memorial Hospital regimen utilize effective antileukemic agents?*—As already discussed (1), the fact that an agent produces pancytopenia is not necessarily an indication that the agent produces an antileukemic effect. On the basis of their documented antileukemic activity when administered as single agents, only two of the agents utilized by Arlin and colleagues (cytarabine and daunorubicin) could be considered to be potent antileukemic agents. The other five agents that were administered during "consolidation" therapy have a low order of activity against ANLL, producing remissions in < 15% of the patients. One might expect that these agents may have produced greater toxic effects on normal hematopoietic elements than on residual leukemic cells. Furthermore, while the administration of cyclophosphamide and carmustine to recruit leukemic cells into cycle is theoretically attractive, is there evidence that such recruitment actually occurred? If so, were the normal resting hematopoietic stem cells also recruited into cycle? If the latter occurred, prolonged and severe pancytopenia would be expected. With so many unanswered questions regarding the protocol employed by Arlin and colleagues and considering the differences in their treatment regimen and in the regimens employed by other investigators, how can one derive general principles on the basis of a single treatment protocol which was unique to Memorial Hospital?

The data provided by Mayer et al (2) and the results of the ongoing Roswell Park study are encouraging and suggest that these two regimens have a lower mortality rate and are more therapeutically effective than the regimen employed at Memorial Hospital. Further patient follow-up and perhaps direct comparative studies will be necessary before definitive conclusions can be drawn regarding the efficacy of these two regimens. Finally, given that relapse occurs for a variety of reasons (3) and that pretherapy in vitro studies are being identified that are capable of indicating which patients are likely to have long or short remissions (4),[1,2] it should now be possible to utilize currently available chemotherapeutic agents on a more rational basis than has been possible in the past and perhaps to administer therapy which might result in the long-term survival of most leukemic patients.

REFERENCES

1. PREISLER HD. Therapy for patients with acute myelocytic leukemia who enter remission: bone marrow transplantation or chemotherapy? Cancer Treat Rep 66:1467–1473, 1982.
2. MAYER RJ, WEINSTEIN HD, CORAL FS, ET AL. The role of intensive postinduction chemotherapy in the management of patients with acute myelogenous leukemia. Cancer Treat Rep 66:1455–1462, 1982.
3. PREISLER HD. Treatment failure in AML. Blood Cells Symposium, 1982. In press.
4. RUSTUM Y, and PREISLER HD. Correlation between leukemic cell retention of 1-β-D-arabinosylcytosine-5′-triphosphate and response to therapy. Cancer Res 39:42–49, 1979.

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[1]Preisler HD, Azarnia N, and Marinello M. Growth of leukemia cells in vitro: relationship to patient and leukemic cell characteristic and to outcome of therapy. Submitted to *Cancer Research*.

[2]Preisler HD, Gessner T, Azarnia N, et al. Relationship between adriamycin plasma levels and the outcome of remission induction therapy for acute nonlymphocytic leukemia. Submitted to *Cancer Chemotherapy and Pharmacology*.

The Ninth Annual Symposium on Diagnosis and Treatment of Neoplastic Disorders — Medical, Surgical, and Radiotherapeutic Aspects

Baltimore, Maryland, April 7–8, 1983

Presented by the Oncology Center of the Johns Hopkins University, at the Johns Hopkins Medical Institutions, this 1983 symposium will cover a wide range of topics including mini-symposia on lung cancer, autologous marrow transplantation in cancer therapy, and Hodgkin's disease, as well as a symposium sponsored by A. H. Robins on chemotherapy-induced nausea and vomiting and its management. The program will also include discussions of the endocrine and sexual consequences of cancer chemotherapy and the management of fibrocystic disease of the breast. In addition, new approaches to cancer therapy such as radioimmunogloblin therapy and new drugs in phase II trials will be discussed. For this program, the Hopkins' faculty will be joined by nine distguished guest lecturers.

This symposium meets the criteria for 15 CME Category I credit hours awarded by the AMA and the fee which includes social activities is $225.

For more information, contact: Program Coordinator, Continuing Education, Turner Auditorium, Rm 22, 720 Rutland Ave, Baltimore, MD 21205 (telephone: 301-955-6046).

International Symposium of Biological and Clinical Aspects of Soft and Hard Tissue Repair

Innisbrook at Tarpon Springs, Florida, May 11–15, 1983

This symposium meets the criteria for 20 credit hours in AMA Category I of the Physician's Recognition Award. Tuition is $225 and the deadline for registration is April 8, 1983. Early registration is advised because enrollment is limited.

For further information, contact: Symposia Medicus, 2880 Shadelands Dr, Suite 404, Walnut Creek, CA 94598 (telephone 415-935-7889).

Thirteenth Annual Neurosurgery Postgraduate Course and the Seventh Annual Neurosurgical Nurses' Conference

San Francisco, California, May 26–27, 1983

Presented and sponsored by the University of California School of Medicine's Department of Neurological Surgery and Extended Programs in Medical Education, this course will provide an update on selected topics in clinical neurosurgery.

Cerebral tumors, spinal injuries and lesions, cerebrovascular disease, and recent clinically relevant advances in the neurologic sciences will be covered in depth. In addition to the didactic presentations, small luncheon discussion sessions will give participants an opportunity in interact with faculty. This program meets the criteria for 15 AMA/CMA Category 1 credit hours.

For further information, contact: Extended Programs in Medical Education, Rm 569-U, University of California, San Francisco, CA 94143 (telephone 415-666-4251).

Taste and Smell

New York, New York, June 2–3, 1983

A conference on taste and smell in humans will be held at Memorial Sloan-Kettering Cancer Center. Clinicians and basic scientists will take part in an exchange which is aimed at improving and increasing the clinical diagnostic evaluation of taste and smell.

For further information, contact: Dr. Richard S. Rivlin, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021 (telephone 212-794-7703).

Predictive Drug Testing on Human Tumor Cells

Zurich, Switzerland, July 20–22, 1983

An international conference on predictive drug testing on human tumor cells will be held at the University Hospital of Zurich. The conference will deal with newly developed techniques for individualizing treatment of cancer patients. The program will include lectures by invited speakers, competitive papers, and a poster session. The deadline for receipt of abstracts is April 15th, 1983.

For further information, contact: V. Hofmann, MD, Division of Oncology, University Hospital, 8091 Zurich, Switzerland.

International Symposium on Detection and Treatment of Minimal Residual Disease in Acute Leukemia

Rotterdam, The Netherlands, October 12–14, 1983

This symposium, co-sponsored by the Comprehensive Cancer Center Rotterdam, focuses on detection and treatment of subclinical disease in acute leukemia. The relative value of chemotherapy and bone marrow transplantation programs as well as the usefulness of new experimental approaches (cell separation, cell culture systems, monoclonal antibodies) and their integration into clinical management will be the major topics. International experts in the field will participate in specific panel discussions on selected subjects. Abstract forms must be received *before July 1, 1983.*

For further information, contact: Dr. J. W. van der Velden, c/o Organization Committee, Leukemia Symposium "Detection and Treatment of Minimal Residual Disease in Acute Leukemia", Dr. Daniël den Hoed Kliniek/Rotterdam Radio Therapeutic Institute, P. O. Box 5201, 3008 AE Rotterdam, The Netherlands.

Current Concepts in Cancer Therapy, II

St. Louis, Missouri, November 3–5, 1983

This meeting will be presented by the Department of Surgery and the Office of Continuing Medical Education, Washington University School of Medicine in St. Louis. The program chairman is Marc K. Wallack, MD. The meeting will be accredited for 17 hours AMA Category 1, AAFP Prescribed, and AOA.

For further information, contact: Loretta Giacoletto, Office of CME, Washington University School of Medicine, Box 8063, 660 S Euclid, St. Louis, MO 63110 (telephone 314-454-3873).

Cincinnati Cancer Conference: Gastro Intestinal Malignancies

Cincinnati, Ohio, November 4–5, 1983

This conference is sponsored by the Bethesda Hospital and Deaconess Association, and the University of Cincinnati.

For further information, contact: Thomas O'Connor, Medical Staff Education, Bethesda Hospital, 619 Oak St, Cincinnati, OH 45206.

International Symposium of the Ludwig Boltzmann Research-Institute for Infections and Tumors of the Urinary Tract

Vienna, Austria, November 25–26, 1983

Symposia topics include: predictibility of cancer chemotherapy with special respect to renal, bladder, prostatic, and testicular tumors; persistent asymptomatic microhematuria; and percutaneous surgery of the kidney—diagnostic and therapeutic approach—indication, techniques, risks, and results.

For further information, contact: Dr. P. Porpaczy, c/o Wiener Medizinische Akademia, Alser Straße 4, A-1090, Vienna, Austria. Telephone: (0222) 42 71 65, Cables: Medacad Wien.

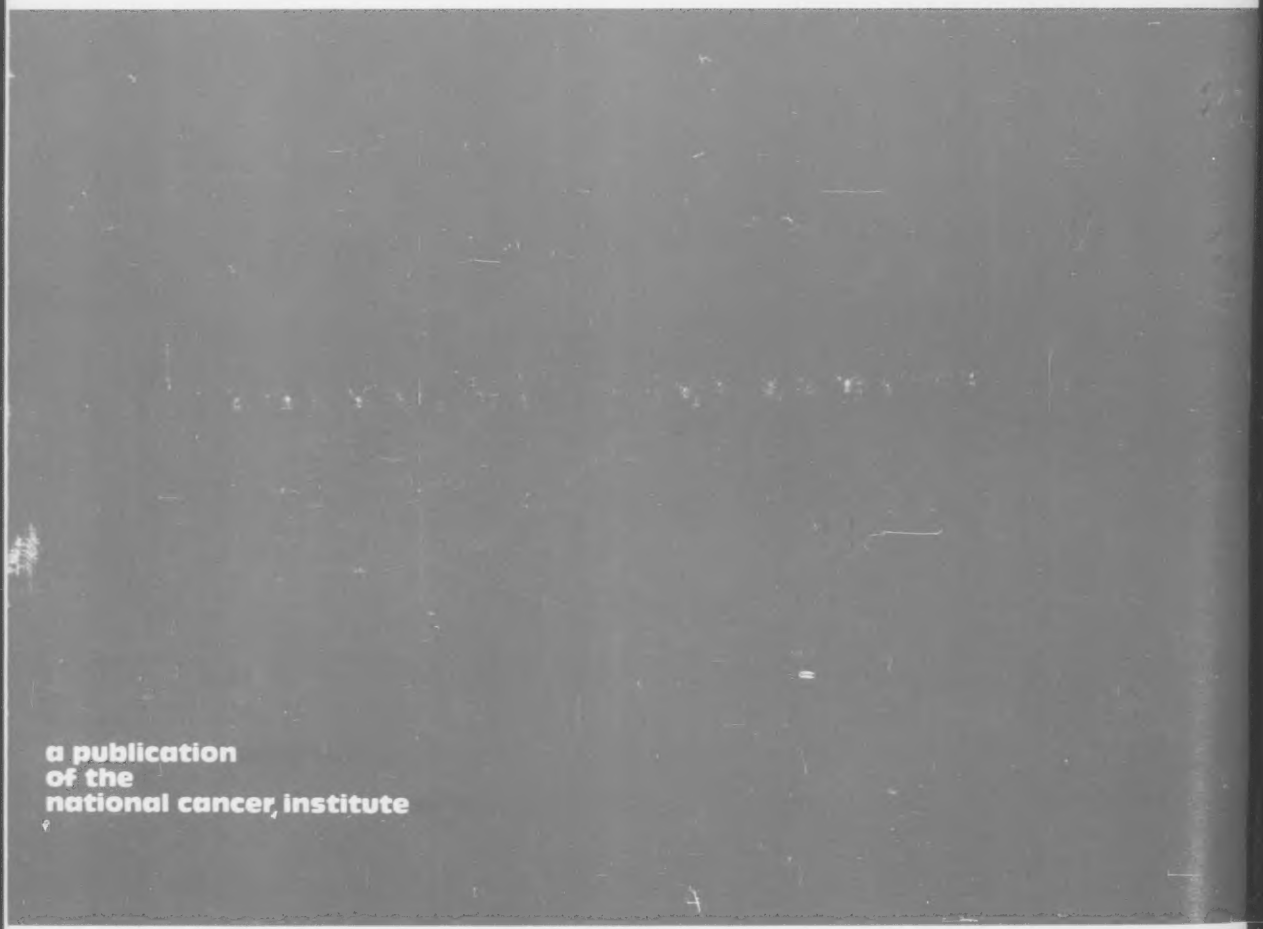
International Conference on Head and Neck Cancer

Baltimore, Maryland, July 22–27, 1984

The American Society for Head and Neck Surgery and The Society of Head and Neck Surgeons are sponsoring this conference to be held at the Hyatt Regency Hotel in Baltimore's Inner Harbor. Topics will include basic research in virology, biochemistry, immunology, cell and tissue culture and other areas; etiologic factors; carcinogens; viral studies; epidemiology; early detection; diagnosis and staging; results of surgery, radiation therapy and chemotherapy; modifications of standard treatments; new treatment modalities; nutrition; rehabilitation; reconstruction; design of clinical trials; and statistical considerations.

The conference will provide dissemination of information through didactic sessions, symposia, panels, workshops, cine discussions, and scientific exhibits. Abstracts are invited for oral presentations and scientific exhibits.

For further information, contact: The Program of Continuting Education, University of Maryland School of Medicine, 10 S Pine St, Baltimore, MD 21201 (telephone 301-528-3956).



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